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Epidemiological aspects of persistent malaria along the river Thenpennai (Tamil Nadu)

S.C. Tewari, N.C. Appavoo*, T.R. Mani, R. Reuben, V. Ramadas* & J. Hiriyan

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Received May 18, 1983

After resurgence of malaria in the mid-seventies in the riverine villages below the Sathanur Dam in Tamil Nadu (India) intensive control measures have brought down the incidence of cases but have failed to interrupt transmission completely. *Plasmodium vivax* is the dominant species of parasite and transmission is perennial, showing two main peaks in March-April and October-November, respectively. Three important potential vector species were found to breed in the river, river-bed pools and streams of the area *viz.*, *Anopheles culicifacies*, *A. fluviatilis* and *A. varuna*. The breeding of *A. culicifacies* was extensive.

The river Thenpennai has its source in Karnataka State, and traverses four districts of Tamil Nadu (Krishnagiri, Dharmapuri, North Arcot and South Arcot) before opening into the sea near Cuddalore. The villages within about 2 km of this river and some of its main tributaries are endemic for malaria for a distance of about 150 km above and below the Sathanur, Dam (Fig.), constituting one of the major problems of persistent malaria transmission in Tamil Nadu at the present time. In this communication salient features of the epidemiology of malaria in this area are reported together with the topography and breeding of anophelines. These constitute the background information for in-depth studies of the anopheline fauna, particularly the recognised vector, *Anopheles culicifacies*, which were undertaken in 1980-81 to identify the reasons for failure to interrupt transmission and to provide the necessary information for the development of a sound strategy of control.

Topography of the area : The Sathanur Dam, built across the river Thenpennai, was completed in 1957. The Dam and the reservoir behind it, are surrounded by scrub jungle intersected by a large number of streams and low rocky hills. Since the terrain here is rugged, a smaller pick-up *anaicut* has been built across the river about 6 km downstream, immediately above which a left bank irrigation canal takes its origin, and a similar right bank canal has recently been completed. A carefully controlled amount of water is let out into the canals from January to May each year for irrigation purposes, while excess water, especially in seasons of heavy rainfall,

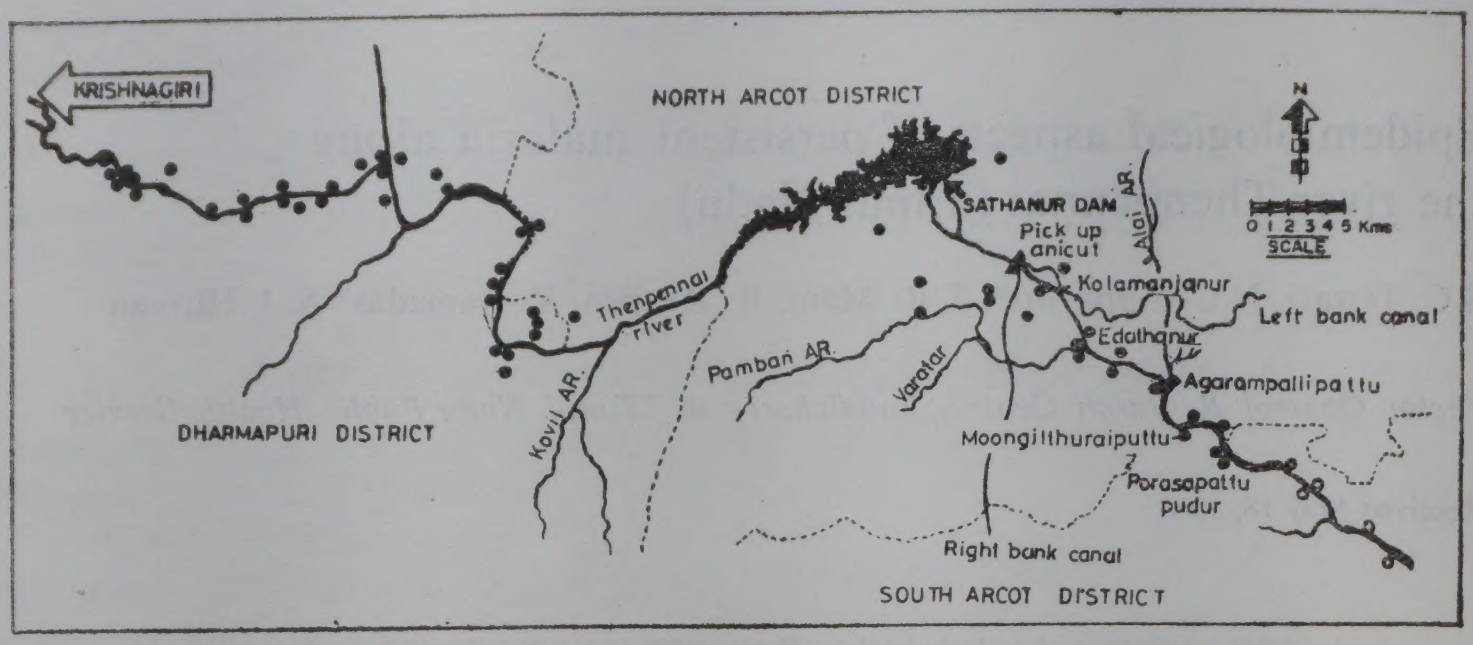

Fig. Map showing Sathanur dam and problem villages of the riverine tract.

is let out through the main channel. The irrigated villages downstream from the Dam grow three paddy crops a year, while villages upstream and further from the canals depend on well irrigation. Paddy planting here is less extensive and usually restricted to one crop a year.

Climate : The area receives some rainfall under the influence of the south west monsoon from May to July, and heavier during October-November under the influence of the north east monsoon. Table I gives the meteorological data measured at Sathanur Dam.

Choice of indicator villages : Four villages of Vanapuram PHC in North Arcot district were selected for study on the basis of high incidence of malaria in the past three years. These villages were: (1) Sathanur Dam (with a population of 935) situated at the reservoir; (2) Kolamanjanur (population = 342); (3) Edathanur (population = 2130); and (4) Agarampallipattu (population = 1562). Two other villages in South Arcot district included in the second year of the study were (5) Moongilthuraipattu (population = 4658) and (6) Porasapattu Pudur (population = 1646). The location of these villages is shown in the Fig.

Material & Methods

Three tours were made to the area during the first six months of 1980 and continuous observations were made from August 1980 onwards. Epidemiological information was obtained from the State Public Health Department, which maintains surveillance for malaria cases. Larval sampling stations were selected in each village so that all types of habitats were sampled roughly in the proportion in which they occurred. More than 12 larval surveys were carried out in a month. Standard dippers were used in all habitats except in wells, where bucket samples were taken. In running water, one dip was taken every meter, while in other habitats 2 to 5 dips were taken, depending on the area of the breeding surface. In paddy fields 20 dips were taken in each irrespective of the size. Larvae were counted in the field and samples were

Table I. Meteorological data recorded at Sathanur Dam

	Mean max. temp. °C		Mean min. temp. °C		Mean RH % (8 am)		Rainfall, mm		No. of rainy days	
	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981
January	33·3	33·9	22·2	22·8	—	84·0	0	13·0	0	2
February	33·3	35·0	17·8	22·8	—	65·0	0	0	0	0
March	35·6	35·6	21·1	22·2	—	67·0	0	69·0	0	3
April	36·7	38·3	24·4	24·4	—	68·0	6·0	17·0	1	2
May	42·2	41·7	29·4	25·6	—	70·0	58·5	191·0	6	10
June	36·1	35·6	24·4	24·4	—	67·5	9·0	24·0	2	1
July	34·4	36·7	24·4	23·3	92·0	76·2	48·5	77·0	8	7
August	35·0	35·0	23·9	24·4	89·0	78·6	72·5	99·0	7	5
September	35·0	33·9	23·9	23·3	91·5	85·6	32·5	207·7	8	12
October	31·1	33·3	24·4	22·2	91·0	90·0	77·5	371·0	6	16
November	32·2	33·3	23·3	21·1	91·0	88·9	133·5	104·0	9	7
December	33·3	32·8	22·2	20·6	91·0	89·0	19·0	31·0	3	7

reared in the laboratory and identified at the adult stage.

Results & Discussion

Malaria and history of control measures : This area was mesoendemic for malaria in the past, and was sprayed with DDT under the NMCP (National Malaria Control Programme) and NMEP (National Malaria Eradication Programme). Decline in incidence of malaria was reported to be slow. Resistance of the vector *A. culicifacies* to DDT was reported by Bhombore *et al*[1] in 1963 but by 1964 cases were no longer reported and subsequently only irregular spraying of receptive areas was carried out[2]. In 1970, cases of *Plasmodium vivax* malaria were detected among immigrant labour working for the consutrction of the right bank canal, and in successive years the numbers of indigenous cases rapidly increased. Regular spray coverage with DDT and BHC was resumed in 1972, but failed to arrest the increase, which escalated to epidemic proportions in 1976. In this year, the Vanapuram PHC, which covers the worst affected villages, downstream from the Dam in North Arcot district, recorded 22,443 cases for a population of approximately 1,40,000 (Table II).

Beginning in 1977, three rounds of spraying with malathion have been carried out every year between May and December by the State Public Health Service. In addition to residual spraying, limited antilarval operations with aromax and temephos are being conducted in the Thenpennai river-bed for approximately 2 km above and below each of the concerned villages, and in 1981, cold aerosol treatment with technical malathion was introduced as a supplement to residual spraying. During 1980 and 1981, two rounds of mass radical treatment were carried out in all the problem villages during the spraying season.

While the malaria incidence has shown a downward trend after the introduction of malathion in 1977 (Table II), transmission persists even after 15 rounds of residual spraying even though the vector was found to be still susceptible in 1982. Constant vigilance thus needs to be maintained to contain localised outbreaks. From an API of 190·1 in 1976, the incidence was reduced to 4·4 in 1980, but rose to 6·6 in 1981, 322 cases being recorded in November of that year. The dominant species of parasite was *P. vivax*, the parasite formula varying from *P. vivax* 99·9 per cent: 0·1 per cent *P. falciparum* in 1975 to 77·5 per cent: 22·5 per cent in 1980. Cases of *P. vivax* as well as *P. falciparum* have been detected in each month, particularly in the epidemic year 1976. However, from 1978 onwards, there was a tendency for two peaks in incidence—one in March-April, before the start of spraying, and the second during the spraying season, in October-November. The API for individual indicator villages was much higher than for the Primary Health Centre as a whole, but seasonal trends in the incidence were the same (Table III), showing that they were representative of the area. Cases have occurred in all age groups, including infants (Table IV). A mass blood survey carried out by our Institute in September 1981 revealed that of 717 blood smears examined, 5 (0·7%) were positive—one for *P. vivax* and four for *P. falciparum*, from persons who were afebrile at the time of obtaining the blood smear.

Table II. Cases recorded at Vanapuram PHC (enumerated population 1,36,907 in 1981)

	1975		1976		1977		1978		1979		1980		1981	
	Pv	Pf+ mx	Pv	Pf+ mx	Pv	Pf+ mx	Pv	Pf+ mx	Pv	Pf+ mx	Pv	Pf+ mx	Pv	Pf+ mx
January	288	0	434	10	2709	230	48	3	25	4	40	4	26	9
February	396	0	589	13	1476	158	53	5	95	4	81	0	24	15
March	1129	0	453	22	669	21	69	12	101	16	68	10	37	21
April	618	0	432	34	790	16	67	8	61	41	93	11	38	15
May	79	0	1603	137	673	4	165	3	76	14	52	4	14	5
June	93	0	1412	99	945	14	118	2	233	20	33	3	32	8
July	657	0	1659	68	1455	17	161	7	26	3	19	0	32	5
August	1443	0	2022	63	918	17	168	7	41	4	21	2	32	3
September	1517	0	1156	90	733	8	229	7	39	2	27	4	51	35
October	690	2	2093	214	234	3	502	1	68	8	38	10	94	23
November	1166	4	5148	555	88	2	66	7	93	45	55	7	274	48
December	951	5	3781	256	33	3	42	2	42	17	13	1	54	19
Total no. cases	9027	11	20882	1561	10715	493	1688	64	900	178	540	56	708	206
Total blood smears examined	24,820		48,415		21,137		22,985		26,901		23,848		26,901	
API (per 1000 population)	80·0		190·1		90·9		13·6		8·2		4·4		6·6	
ABER	21·9		38·5		13·3		14·7		15·0		14·1		16·8	
SPR	36·4		46·3		53·0		7·6		4·0		2·5		2·6	

Table III. Incidence of malaria in six indicator villages in North and South Arcot districts

	North Arcot district								South Arcot district				Total	
	Sathanur Dam		Kolamanjanur		Edathanur		Agarampal-lipattu		Moongilthurai pattu		Porasapattu pudur			
	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981
January	5	0	0	0	1	1	1	1	10	7	2	2	19	11
February	2	3	0	0	3	1	1	1	8	4	8	3	22	12
March	5	2	0	1	6	5	10	3	36	21	63	0	120	32
April	7	2	2	8	12	3	14	3	17	18	2	1	54	35
May	4	2	0	2	2	0	2	0	11	3	3	2	22	9
June	0	8	0	0	3	0	2	1	9	4	21	3	35	16
July	0	5	0	1	0	2	0	0	6	12	2	3	8	23
August	1	1	0	1	0	0	1	1	10	9	3	1	15	13
September	0	22	0	1	0	4	4	2	18	5	10	3	32	37
October	3	16	0	2	1	8	9	6	14	15	4	4	31	51
November	1	26	1	2	4	27	0	25	18	19	32	9	46	108
December	0	14	0	3	0	9	1	2	7	15	8	4	16	47
API (per 1000 population)	30·0	105·9	6·1	42·7	15·0	25.4	28·8	28·8	35·2	28·3	89·9	21.3	—	—

Table IV. Age-wise distribution of malaria cases for 1980-81 at Vanapuram Primary Health Centre

Year	Species	Age group					Total	Male	Female
		<1	1-4	5-9	10-14	15+			
1980	Pv	—	54	79	75	332	540	301	239
	Pf	1	3	7	10	34	55	32	23
	Mixed	—	—	—	—	1	1	1	—
1981	Pv	4	76	108	100	420	708	399	309
	Pf	—	12	25	51	99	187	103	84
	Mixed	—	1	2	4	12	19	7	12

Population movements : While indigenous domiciliary transmission occurs in the whole riverine tract, Sathanur Dam in particular is a focus for extradomiciliary transmission from which the disease is disseminated by population movements. Hyma and Ramesh[3] have pointed out that thousands of tourists visit the area. However, since the vast majority of these are day-trippers, who leave when the park gates close at 7·30 pm, we believe that these authors have given an exaggerated importance to the tourists. Of greater epidemiological significance is the annual migration of cow-herds from Thanjavur and Ramnad districts, who bring in October each year, large numbers of cattle and goats to graze in the jungle round the Dam. These cow-herds remain in the area until December, either sleeping in the open or in temporary unsprayed huts. Others spending the night in the jungle close to the river are the night-duty staff of the Dam, stone-cutters and other immigrant workers, as also illegal wood-gatherers and fishermen. One illegal fishing party of 52 persons spent 20 days in June camping by the river. Six persons subsequently developed malaria on returning to their homes in distant villages. Many such instances of extra domiciliary transmission occur.

Anopheline breeding : Eleven anopheline species were identified from larvae collected from natural habitats (Table V). Of these, *A. subpictus* was the dominant species in all habitats with standing water, *A. culicifacies* being second. In flowing water, however *A. culicifacies* predominated. Riverbed-pools accounted for 65·9 per cent of all the larvae of *A. subpictus* which were collected, while 24·8, 2·4 and 0·8 per cent were collected in paddy fields, rivers and streams respectively. *A. culicifacies* bred extensively in riverbed-pools (51·4%), while 36·8 and 6·9 per cent were collected in the slow flowing water at the grassy edges of the river and streams respectively. Very few were found in paddy fields (3 out of 3554 larvae identified from 171 fields). *A. fluviatilis* and *A. varuna* were found primarily in streams, but a few were found in pools and in the main river also, and four specimens of *A. fluviatilis* were obtained from a well.

Table V. Anopheline species identified from various larval habitats

Habitat	No. of larvae of each species identified*								
	A. (A) barbirostris gr.	*A. (A) hyrcanus* gr.	*A. (C) annularis*	*A. (C) culicifacies*	*A. (C) fluviatilis*	*A. (C) pallidus*	*A. (C) subpictus*	*A. (C) vagus*	*A. (C) varuna*
Slow flowing river water	10	10	169	1302	7	9	209	6	11
River-bed pools	13	2	66	1914	21	20	7714	63	7
Streams	30	3	31	244	68	11	89	4	88
Canals	0	0	33	48	0	1	84	3	0
Wells	2	0	0	22	4	0	10	2	0
Cement tanks	0	0	0	6	0	0	261	1	0
Rainwater pools	0	4	5	0	0	6	8	2	0
Paddy fields, $<6''$	0	6	4	3	0	24	2904	185	0
Paddy fields, $>6''$	0	35	7	0	0	31	338	17	0
Total	55	60	315	3539	100	102	11698	283	106

**A. jamesi* and *A. maculatus* were also recorded in very small numbers in streams and in riverbed pools

The river and its associated riverbed-pools were a perennial source of breeding, accounting for 71·9 per cent of all the anopheline larvae collected. The average number of pools varied from 42 to 153 per km of riverbed in different months (58 to 153 m^2 available breeding surface). The number was highest from January to March due to spillage of water from the Dam, low in May and again high from August to November due to rain water. The number of larvae collected per positive dip varied in different months (Table VI) but no clear seasonal variations could be detected due to the antilarval operations in progress. In seasons of heavy rain fall, breeding was sometimes washed out for a few days. Breeding was detected in the streams in all seasons, but many dried out completely during the hot months.

The construction of the Sathanur Dam has afforded additional breeding sites for *A. culicifacies* by slowing down the current in many places and exposing the irregular rocky river-bed in which pools form. Many breeding sites are virtually inaccessible because of the rugged terrain. The increased acreage under paddy has not increased the vector population, since the species was rarely found in this habitat. However, intense breeding may take place in temporary pools in the irrigation canals immediately after the water is shut off, and one of the localised outbreaks of malaria has been attributed to this cause (unpublished data, NMEP). There is also breeding in rain water pools in the canals during the non-irrigation season. Nevertheless, the main problem along the Thenpennai river is of true riverine malaria, irrigation playing a comparatively minor role. This is in contrast to the situation that prevailed in Pattukottai in the late thirtees and early forties, where breeding was predominantly in the irrigation channels, borrow pits and paddy fields, and negligible in the river Cauvery[4]. Even today, the species is to be found in the same habitats, though the extent of breeding in the paddy fields has considerably diminished[5,6].

Table VI. Number of larvae per positive dip in various habitats

Month (1981)	Habitat						
	River	Riverbed pools	Streams	Canals	Paddy fields		Misc.
					<6″	>6″	
January-February	9·7	10·4	4·9	—	7·8	5·6	3·3
March-April	2·4	8·3	3·2	—	5·7	4·6	6·0
May-June	3·7	7·5	3·4	12·0	2·9	—	2·2
August	6·2	13·8	4·0	0·0	23·6	18·5	2·2
September	7·7	20·5	3·4	0·0	21·4	9·4	2·5
October	4·1	12·8	3·3	2·8	15·5	2·8	2·7
November	2·1	23·2	2·2	2·2	8·1	3·0	4·5
December	3·0	5·5	6·1	0·0	—	—	1·5

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Resting behaviour of anophelines & sporozoite rates in vectors of malaria along the river Thenpennai (Tamil Nadu)

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Anopheles culicifacies in high densities was captured resting outdoors as well as indoors in riverine villages of Tamil Nadu where malaria is persistent. A sporozoite rate of 0·6 per cent was obtained for this species, and no other species could be incriminated as a vector. While the outdoor resting population comprised mainly males and newly emerged females, some physiologically aged and potentially dangerous females were also found. It was concluded that the large numbers of human-fed and sporozoite positive female mosquitoes resting indoors were mainly responsible for continued transmission of malaria, while outdoor resting vectors also play a role in transmission.

The resting behaviour of vectors of malaria is an important factor in the success or failure of control programmes employing indoor residual spraying with insecticides. Most anophelines rest outdoors to some extent, but if a species is wholly or largely exophilic, a large proportion of the mosquito population will escape contact with the insecticide and transmission will not be interrupted. A detailed investigation of this aspect of vector behaviour was undertaken in riverine villages of Tamil Nadu where it has been shown that both domiciliary and extra-domiciliary transmission of malaria take place[1].

Material & Methods

The area and the indicator villages chosen for study have been described elsewhere[1]. Adult mosquitoes were collected with suction tube and torch for 15 min per structure in houses and cattlesheds selected at random. Natural outdoor resting places were also sampled on a timed basis with suction tube and torch. In addition, 4-6 pit traps (1 × 1 × 1 m) were dug in each indicator village and visited at least three times in a month. The pits were watered by local villagers the evening before the collections were due, to make them more attractive to mosquitoes.

The anophelines captured were classified by species and sex, and the females were analysed by their abdominal condition. They were dissected and examined for the presence of the malarial parasite on the day of capture, or the day after. As the guts of freshly fed females were smeared on filter paper for determina-

tion of the source of the bloodmeal, fewer guts were examined than glands. Polodovova's technique of counting dilatations on the ovariole, in combination with Detinova's tracheolar method, was used to determine the physiological age.

Results

Indoor resting density : Of the nine species of anophelines captured resting indoors, *Anopheles subpictus* was the most abundant, *A. culicifacies* being next in abundance; while *A. fluviatilis* and *A. varuna* were rarely encountered (Table I). *A. culicifacies* usually rested deep in the thatch, in dark corners and cracks, but also in relatively exposed situations on damp, newly constructed mud wells, often at ground level.

The per man-hour density of *A. culicifacies* was relatively low from January to March, but high in the later months of the year (Table I), in spite of three rounds

Table I. Captures of indoor resting anophelines

Month	No. of man hours	No. captured per man-hour			
		A. culicifacies	*A. subpictus*	*A. vagus*	*A. varuna*
1980 :					
Jan.-Feb.	51·00	1·12	15·64	3·39	0
March-April	68·75	1·05	44·90	2·82	0·03
May-June	73·50	1·76	0·37	0	0
August	49·00	2·92	17·27	0·18	0
September	48·50	4·47	15·59	0·93	0
October	50·75	3·67	26·72	1·56	0
November	48·00	4·19	18·38	0·83	0·02
December	56·75	2·10	4·23	0·02	0
1981 :					
January	69·75	1·98	9·30	0	0·01
February	92·25	3·12	32·63	0·08	0·01
March	96·00	3·58	24·48	0·06	0·01
April	96·00	5·95	15·32	0	0·02
May	104·00	5·64	2·66	0·01	0·01
June	94·00	8·36	13·28	0·01	0·01
July	97·75	4·57	30·14	0·02	0
August	101·25	5·56	9·14	0·09	0·04
September	96·00	7·77	25·63	0·08	0·02
October	96·00	9·75	25·93	0·18	0·01
November	94·50	7·30	18·74	0·28	0·03
December	96·00	17·61	8·13	0·30	0·03

Species captured in very small numbers were *A. hyrcanus*, *A. annularis*, *A. fluviatilis*, *A. pallidus* and *A. tessellatus*

of residual spraying carried out annually between May and December. Densities were higher in 1981 as compared to 1980, which may be related to the much heavier rainfall in the second year of the study[1]. *A. subpictus* showed three peaks in abundance, *viz.*, February-April, July and September-October respectively, corresponding to the main paddy planting seasons.

Outdoor resting density: Outdoor resting was detected in all the indicator villages. In 588 man-hours of collecting, 2348 *A. culicifacies*, 319 *A. subpictus*, 202 *A. varuna*, 25 *A. fluviatilis*, 6 *A. vagus*, 5 *A. pallidus*, 3 *A. tessellatus*, 1 *A. barbirostris* and 1 *A. jamesi* were collected from holes in damp mud banks by the river, crevices between stones, palmyra leaf fences and a culvert. The culvert was highly productive, up to 400 *A. culicifacies* per man hour being obtained there.

Systematic sampling by means of pit traps showed that outdoor resting of *A. culicifacies* was high in January-February, and again in November 1980. In 1981, maximum numbers were captured in June and July (Table II). *A. subpictus* was most numerous in the pit traps in the same months as it dominated in the indoor collections also. *A. fluviatilis* was captured in good numbers in the initial months of the study, after which *A. varuna* was much more abundant.

Analysis of the indoor and outdoor resting populations: The sex ratio of catches from different habitats together with classification of females by abdominal condition is shown in Table III. Males of *A. culicifacies* were scarce indoors (male : female ratio 1 : 5·1 indoors as compared to 1 : 1·2 and 1 : 1·4 outdoors). Unfed females constituted only 5·7 per cent of the catch indoors, but 17·3 and 25·2 per cent outdoors. The numbers of fully fed females were roughly equal to those of semigravid and gravid females indoors and in natural outdoor habitats, but semigravid females predominated in the pit trap collections. Since males of *A. fluviatilis* and *A. varuna* could not be distinguished, except by association with females in the same collections, catches of these species were combined to obtain male : female ratios of 1 : 1·9 and 1 : 2·4 in natural outdoor resting places and pit traps respectively.

The age composition of the indoor resting population of *A. culicifacies* and those resting in natural outdoor habitats was very similar (Table IV) with 0·70 and 0·65 of the population respectively being parous. Aged females with up to 4 dilatations in the ovariole were recorded from both. Females from pit trap collections were predominantly nulliparous, only 0·18 showing one or two dilatations.

Natural infection rates: Thirteen sporozoite positive specimens of *A. culicifacies* were obtained out of 2172 glands examined (0·6%). All these were from indoor resting collections, except one which was captured from human bait indoors. None of 387 specimens dissected from outdoor habitats was positive. Higher sporozoite rates (up to 2·7%) were obtained in the earlier months, as compared to the later months of the study (Table V) corresponding to high incidence of malaria[1] during March and April, 1980.

No positive *A. fluviatilis* was obtained out of 63 guts and 112 glands examined. One oocyst positive specimen of *A. varuna* was detected out of 69 guts examined, but there was no sporozoite positive out of 951 glands.

Table II. Captures of outdoor resting anophelines in pit shelters

Month	No. of trap/ nights	No. captured per 10 traps/night				
		A. culicifacies	*A. fluviatilis*	*A. subpictus*	*A. vagus*	*A. varuna*
1980 :						
Jan-Feb.	70	8·00	4·43	7·43	23·14	0
Mar-Apr.	85	2·82	9·06	11·06	4·59	4·59
May-June	83	1·33	0	0	0	0·48
August	47	2·13	0	1·70	0	0·64
September	50	3·60	0	2·00	0·40	2·60
October	55	4·18	1·53	7·12	2·37	4·24
November	59	7·46	0·34	0·85	0·34	4·92
December	60	5·17	0	0·67	0	12·83
1981 :						
January	85	4·00	0	0·47	0·12	7·53
February	101	10·20	0	11·78	5·94	12·77
March	107	1·68	0	19·25	0·65	7·10
April	102	3·13	0·10	2·55	0	7·16
May	83	4·58	0	1·08	0	2·89
June	79	26·20	0·25	4·81	0·63	16·96
July	82	18·78	0	6·34	0·37	30·61
August	84	11·19	0·36	1·43	0·36	23·81
September	83	7·83	1·20	2·17	0·60	11·20
October	79	3·42	0	3·16	4·30	15·57
November	64	0·02	0	3·43	3·58	4·22
December	81	1·98	0·25	1·60	2·96	7·41

Other species captured in pit traps were *A. hyrcanus, A. annularis, A. jamesi, A. karwari, A. maculatus A. pallidus* and *A. tessellatus*

Discussion

A. culicifacies was clearly the vector responsible for malaria transmission in the riverine tract. *A. fluviatilis* and *A. varuna* could not be implicated in this study. In the past sporozoite rates of *A. culicifacies* have tended to be low in endemic situations, being only 0·061 per cent in Pattukottai, Thanjavur district[2]. The value of 0·6 per cent obtained here is relatively high, though much higher rates have been recorded during epidemics *i.e.*, 13 and 31 per cent in Sind and Mysore respectively[2,3].

Indoor resting densities recorded in southern India in the post-DDT era have generally been low—up to 1·9 per man hour in Pattukottai[4] and low also in Rameswaram Island (unpublished data from VCRC). Thus, the high indoor resting densities observed in the Sathanur area, up to 17 per man-hour, together with the high level of outdoor resting detected,

Table III. Captures of anophelines from various habitats analysed by sex and abdominal condition of females

Species	Habitat	Total no.		Sex ratio	% of females			
		M	F		UF	FF	SG	G
A. culicifacies	Indoors	184	941	1 : 5·1	5·7	47·6	45·8	0·9
	Outdoors	204	260	1 : 1·3	17·3	48·1	34·2	0·5
	Pit traps	90	127	1 : 1·4	25·2	29·9	43·3	1·6
A. subpictus	Indoors	1680	6313	1 : 3·8	5·5	22·2	66·4	5·8
	Outdoors	14	30	1 : 2·1	20·0	3·3	76·7	0·0
	Pit traps	100	50	2 : 1	52·0	16·0	32·0	0·0
A. fluviatilis	Indoors	0	4	—	—	—	—	—
	Outdoors	6	12	—	16·7	58·3	25·0	0·0
	Pit traps	44	43	—	39·6	30·2	28·0	2·3
A. varuna	Indoors	0	3	—	—	—	—	—
	Outdoors	49	93	1 : 1·9*	15·1	44·1	40·9	0·0
	Pit traps	38	152	1 : 2·4*	25·7	52·0	24·4	0·0

*Combined sex-ratios for *A. fluviatilis* and *A. varuna*

Table IV. Age composition of day-time resting populations of *A. culicifacies*

Habitat	No. dissected	No. of dilatations					Proportion parous
		0	1	2	3	4	
Indoors	2005	601	1278	112	11	3	0·70
Outdoors	265	93	155	12	4	1	0·65
Pit traps	185	151	33	1	0	0	0·18

Table V. Summary of dissections for the presence of the malarial parasite

Period	Guts		Glands	
	No. +ve/ dissected (%)		No. +ve/ dissected (%)	
A. culicifacies				
Oct-Nov. 1979	1/82	(1·2)	1/81	(1·2)
Jan-Feb. 1980	4/287	(1·4)	4/343	(1·2)
March-April	2/87	(2·3)	5/185	(2·7)
May-June	0/97		0/167	
August	0/98		2/253	(0·8)
September	0/75		0/221	
October	0/91		1/245	(0·4)
November	0/222		0/504	
December	0/56		0/173	
Total October 79-December 80	7/1095	(0·6)	13/2172	(0·6)
A. fluviatilis				
Oct 79-Dec. 81	0/63		0/112	
A. varuna				
Oct 79-Dec. 81	1/69	(1·4)	0/951	

indicate higher than average vector populations in this area.

There are several records of outdoor resting by *A. culicifacies*. The species was captured under a culvert[5], between stones and below bushes[6] in Pune, along canal banks in the Singhbhum and Jeypore Hills[7] and in holes in river banks in Sri Lanka[8]. The species could be trapped in earth lined boxes placed outdoors[9], and in pit traps in Gujarat[10], while relatively large numbers could be captured outdoors in villages in Alwar[11]. However, outdoor resting was rarely detected in Pakistan[12]. It has been stated that where the species is mainly endophilic, the proportion of semigravid and gravid females will be almost the same as that of freshly fed females, whereas if outdoor resting is common, there will be a deficit of semigravids and gravids indoors[9]. In the present study, however, freshly fed and semigravid females occurred indoors in almost equal numbers, suggesting that those females which rested indoors after feeding remained there throughout the gonotrophic cycle.

The presence of a large proportion of males, unfed and nulliparous females outdoors suggests that these sections of the population are mainly exophilic. Shalaby[10] had recorded a high proportion of males and unfed females in pit trap collections of *A. culicifacies*, and there

was indirect evidence that younger females were resting outdoors in Pakistan[13]. While no sporozoite positive females could be found outdoors in the present study, physiologically aged females were represented in the outdoor catches, and 0·057 of the blood fed specimens from this habitat had fed on man[14]. Thus, some epidemiologically dangerous females rest outdoors, and outdoor resting is one of the causes of continued transmission. The major cause however, is the large number of females, many human-fed and sporozoite positive, resting indoors in spite of residual spraying with malathion. The species was susceptible to malathion when tested in July 1982; we will be discussing the effect of control measures in a separate communication.

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Host selection patterns in anophelines in riverine villages of Tamil Nadu

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In a mixed population of sibling species of *Anopheles culicifacies*, species A was relatively anthropophilic (0·214 man feedings), in comparison with species B (0·018). The overall human blood index was 0·118, but there were marked seasonal variations in the proportion of man feedings. *A. fluviatilis* and *A. tessellatus* had indices of 0·026 and 0·021 respectively, while no man feeds were obtained for *A. subpictus*, *A. vagus* and *A. varuna*.

Human blood indices of Indian anophelines were widely studied before the commencement of the National Malaria Eradication Programme[1]. However, relatively few studies have been carried out during and after control, particularly in south India. We report here the results of determination of blood meals of anophelines in an area of persistent malaria transmission, which has been under malathion spray, since 1977.

Material & Methods

Mosquitoes were captured from indoor and outdoor diurnal resting places in six indicator villages which have been described earlier. The mosquitoes were collected in the morning and carried back to the laboratory, usually on ice. The stomach bloods of freshly fed females were smeared on filter paper and sent, by arrangement with the WHO, to the Reference Laboratory at Silwood Park, Ascot, UK, for determination of the blood meal by precipitin test.

Cytological determinations of sibling species were carried out on some of the *Anopheles culicifacies* females. For this purpose, the females were kept at room temperature until the ovaries had developed to Christopher's stage III (early) and then dissected. The stomach blood was smeared immediately and ovarian polytene chromosome preparations were made by standard methods[2].

Results & Discussion

A total of 4250 blood meals were tested from *A. culicifacies* females captured in various habitats. Among those collected indoors, 393 from houses (0·134) had fed on man, while 53 (0·098) from animal dwellings, 6 (0·353) from mixed dwellings, 8 (0·079) from vacant houses and 15 (0·097) collected from walls

during the night, were also positive for human blood. In addition, 29 specimens (0·057) collected from outdoor shelters had fed on human hosts. The overall human blood index was 0·118 (Table I). The cattle-man ratio in this area is 1 : 2·6, although individual villages varied from 1 : 1·2 to 1 : 10·6 (Table II). The HBI in individual villages also varied, from 0·172 in Sathanur Dam to 0·025 in Porasapattu-Pudur (Table II), the three upstream index villages (Sathanur Dam, Kolamanjanur and Edathanur) showing a higher proportion of man-feedings than the three villages further downstream. The human blood index for *A. culicifacies* in Pattukottai, Thanjavur district, was reported[3] to be as low as 0·025, while at Ennore[4], on the coast, in the absence of cattle, it was as high as 0·80. Recently, a high HBI index (0·629) has also been reported from Rameswaram island[5], where the cattle : man ratio is low.

It has been shown[6] that the *A. culicifacies* population of the riverine tract is a mixture of two sibling species—A and B. During this study 434 determinations were made of blood meals from females chromosomally identified as *A. culicifacies* sp. A, of which 0·214 had fed on man (Table I). On the other hand, of the 398 females chromosomally identified as species B, only 0·018 had fed on man. This is the first time that blood meal determinations were carried out for chromosomally identified sibling species of *A. culicifacies*. It is interesting to note that, in Pakistan, where until recently only sp. A had been identified, only 0·053 man feedings were identified in 1976-77 for a population of *A. culicifacies*[7], whereas in Sri Lanka, where only sp. B has so far been reported, between 0·232 and 0·658 man feedings were reported[8] in an area sprayed earlier with DDT. In the Thenpennai riverine tract, where both species were present, sp. A was relatively anthropophilic as compared to the strongly zoophilic sp. B. The difference was maintained in all seasons.

Month-wise analysis revealed a marked seasonal variation in the proportion of man feedings. It was high from February to July (0·170 to 0·426). A declining trend was observed from August onwards, reaching as low as 0·006 in December (Table III). These trends were observed in both years of the study, although numbers tested in 1980 were small. This seasonal change in feeding behaviour is not related to the spraying season, which extends from the end of April to the beginning of December, nor is it related to any change in the relative availability of hosts. A similar reduction in the proportion of man-feedings has been observed during the cold months in Pakistan, when villagers slept indoors under quilts, as compared with the summer months when they slept outdoors[9]. In the riverine villages the period of high man-feeding corresponds to the period when the villagers sleep outdoors, as well as to the season when the more anthropophilic *A. culicifacies* sp. A is dominant; the more zoophilic sp. B has been shown, on the other hand, to predominate in the monsoon and post-monsoon seasons[5], when the villagers sleep mainly indoors. Both the factors perhaps contribute to the shift in the feeding pattern with season.

The other five anopheline species tested were all strongly zoophilic. Only one specimen each of *A. fluviatilis* and *A. tessellatus* had fed on man (Table I). It

Table I. Results of precipitin tests carried out on stomach bloods of wild caught anophelines

Habitat	Total no. tested	Result					
		Man	Bovid	Sheep/ goat	Dog	Pig	Mamm.
A. culicifacies (all)							
Indoors	3745	475	3184	19	8	10	49
Outdoors	505	29	471	2	0	0	3
Proportion		0·118	0·860	0·005	0·002	0·002	0·012
A. culicifacies sp. A							
Indoors	369	88	269	1	1	1	9
Outdoors	65	5	59	1	0	0	0
Proportion		0·214	0·756	0·005	0·002	0·002	0·021
A. culicifacies sp. B							
Indoors	363	6	347	2	0	3	5
Outdoors	35	1	33	0	0	0	1
Proportion		0·018	0·955	0·005	0	0·008	0·015
A. fluviatilis							
Indoors	13	0	13	0	0	0	0
Outdoors	26	1	24	1	0	0	0
Proportion		0·026	0·949	0·026	0	0	0
A. subpictus							
Indoors	939	0	934	0	0	3	2
Outdoors	3	0	3	0	0	0	0
Proportion		0	0·995	0	0	0·003	0·002
A. tessellatus							
Indoors	1	0	1	0	0	0	0
Outdoors	47	1	43	0	0	1	2
Proportion		0·021	0·917	0	0	0·021	0·042
A. vagus							
Indoors	125	0	125	0	0	0	0
Outdoors	4	0	4	0	0	0	0
Proportion	0	0	1·0	0	0	0	0
A. varuna							
Indoors	16	0	16	0	0	0	0
Outdoors	321	0	317	0	0	0	4
Proportion		0	0·988	0	0	0	0·012

Table II. Man-cattle ratio and human blood index of *A. culicifacies* in index villages

Village	Human population	Cattle population	Ratio cattle : man	No. smears tested	Human blood index
Sathanur Dam	935	97	1 : 9·6	870	0·172
Kolamanjanur	342	288	1 : 1·2	2435	0·113
Edathanur	2130	1522	1 : 1·4	390	0·133
Agarampallipattu	1562	1330	1 : 1·2	390	0·059
Moongilthuraipattu	4658	440	1 : 10·6	61	0·060
Porasapattu-Pudur	1646	603	1 : 2·7	122	0·025
Total	11273	4280	1 : 2·6	4268	0·118

Table III. Proportion of *A. culicifacies* feeding on man in different months (January 1980 to January 1982)

	Indoor		Outdoors		Total	
Month	Total no. tested	Proportion +ve man	Total no. tested	Proportion +ve man	Total no. tested	Human blood index
January	424	0·009	13	0·0	437	0·009
February	110	0·309	21	0·048	131	0·267
March	151	0·238	19	0·053	170	0·218
April	238	0·298	30	0·233	268	0·291
May	208	0·356	44	0·091	252	0·310
June	342	0·225	176	0·063	518	0·170
July	139	0·460	16	0·125	155	0·426
August	221	0·131	64	0·016	285	0·105
September	387	0·096	21	0·0	408	0·091
October	464	0·045	83	0·012	547	0·040
November	407	0·025	63	0·0	470	0·021
December	515	0·004	26	0·038	541	0·006

has been suggested that there are two forms of *A. fluviatilis*—an anthropophilic vector race and a zoophilic non-vector race[10]. If this hypothesis is correct, it is the latter which is present in the riverine villages. No bird feedings were recorded although tests for bird blood were included in the study. The small proportion of pig feedings recorded for *A. subpictus* is interesting in view of the fact that Japanese encephalitis virus has been isolated from this species in nature[11], and pigs are well known as animal reservoirs of this virus.

Since the completion of the study reported here, two new sibling species of *A. culicifacies viz.*, C and D have been recognized in the area. Species 'A' and 'B' in this communication, therefore, refer to individuals with A and B type X chromosomes respectively.

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Feeding behaviour, age structure & vectorial capacity of *Anopheles culicifacies* Giles along the river Thenpennai (Tamil Nadu)

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Feeding of *A. culicifacies* on human bait at a village in the Thenpennai riverine tract was relatively high, *i.e.* up to 43 in a single night on a man indoors and up to 32 outdoors. The ratio of the captures on 2 men : 1 calf was 1 : 1·79. Almost 30 per cent of the females captured at bait had ovaries at a late stage of development, indicating a high degree of refeeding within the same gonotrophic cycle. Daily survival rates ranged from 0·74 in the hot dry months to 0·89 in the cool wet months. Vectorial capacities up to 2·914 were calculated for *Plasmodium vivax* and 2·5937 for *P. falciparum*. Relatively high values were obtained in all months.

The capacity of a vector to transmit malaria depends on several factors, which have been given mathematical expression in the *Vectorial Capacity* of Garrett-Jones[1]. Important among these factors are feeding behaviour and longevity, which may vary depending on local conditions. These aspects of vector ecology were therefore studied with special reference to their effect on the transmission potential of *Anopheles culicifacies*—the only species of anopheline which has been implicated as a vector in the Thenpennai riverine tract.

Material & Methods

All-night biting collections were made at fortnightly intervals at the village Kolamanjanur. Torches were covered with red cellophane to give a dim light. Simultaneous collections were made on a man outdoors, a man indoors and a calf in an open shed. Hourly captures were kept separately, and the mosquitoes were identified and dissected next morning. The development of the ovary according to Christopher's classification was noted and the physiological age determined by a combination of Detinova's tracheolar coiling method and Polodovovna's dilatations method[2].

In order to obtain a larger number of dissections for age determination each month, the biting captures were supplemented by captures of freshly fed female *A. culicifacies* resting on cattleshed walls, thatch, *etc.*, during the night and at dawn.

Daily survival rates were calculated from the n^{th} root of the proportion parous,

where n is the gonotrophic cycle in days (Davidson's method). The vectorial capacity was calculated according to the formula of Garrett-Jones, as applied by Molineaux and Gramiccia[3].

Results & Discussion

Feeding behaviour : During the course of 41 all-night biting collections carried out in 1980-81, 674 anophelines belonging to five species were captured on man, of which 80·4 per cent were *A. culicifacies* and 18·5 per cent were *A. subpictus* (Table I). Of the 8554 anophelines belonging to 8 species captured on a calf, 74·6 per cent were *A. subpictus* and 11·4 per cent *A. culicifacies*. The numbers of *A. culicifacies* captured in different hours of the night were analysed. Since no marked shifts in feeding time were observed, the data for all months have been combined and shown in Table II. Peak biting activity was observed between 2100 and 2300 and again between 2400 and 0200. Most of the biting took place before midnight on human bait (68·7 and 64·2 % of the total catch on man indoors and man outdoors respectively), but on the calf 55·6 per cent of the total catch was obtained after midnight.

Table I. Total numbers of anophelines captured on 2 human : 1 calf bait in 41 simultaneous all night biting collections

Species	No. captured on	
	2 men	1 calf
A. hyrcanus gr.	1	140
A. barbirostris gr.	0	20
A. annularis	0	15
A. culicifacies	542	972
A. pallidus	0	108
A. subpictus	125	6384
A. vagus	5	899
A. varuna	1	16
Total	674	8554

Up to 43 *A. culicifacies* could be captured in a single night on human bait indoors, and up to 32 outdoors. During the first 8 months of the study, captures on human bait indoors were consistently higher than captures outdoors. Subsequently, captures outdoors were sometimes higher and sometimes lower than the indoor captures, without any clear relationship with spray dates (Fig.). Over the whole period of the study, the number of *A. culicifacies* captured on human bait indoors was only slightly higher than the total number captured on human bait outdoors, and the ratio of those captured on 2 men : 1 calf was 1 : 1·79.

When captures of *A. culicifacies* at bait were sorted by abdominal condition, it was found that 22·4 per cent of the total capture on human bait and 38·4 per cent of the captures on the calf bait, were either semigravid or gravid at the time of capture. Refeeding within the same gonotrophic cycle was further confirmed by dissection results (Table III) showing that a high proportion of females with ovaries at Christopher's stages 4 and 5 are captured at bait. Since many of these females were captured immediately after alighting, it is not certain that all of them would have fed, but at least some females with ovaries at stages 4 and 5 were found to contain fresh blood in the stomach. Nulliparous females with ovaries at stage 1 representing females taking their first

Table II. Time of capture of *A. culicifacies* on human and animal bait

Time	Bait			Total	%
	Man indoors	Man outdoors	Calf open shed		
1800-1900	12	13	37	62	4·10
1900-2000	22	21	53	96	6·34
2000-2100	43	33	67	143	9·45
2100-2200	36	41	102	179	11·82
2200-2300	60	44	84	188	12·42
2300-2400	25	11	88	124	8·19
2400-0100	22	28	115	165	10·90
0100-0200	21	23	104	148	9·78
0200-0300	14	7	71	92	6·08
0300-0400	13	2	104	119	7·86
0400-0500	8	7	59	74	4·89
0500-0600	12	24	88	124	8·19
Total	288	254	972	1514	100·02

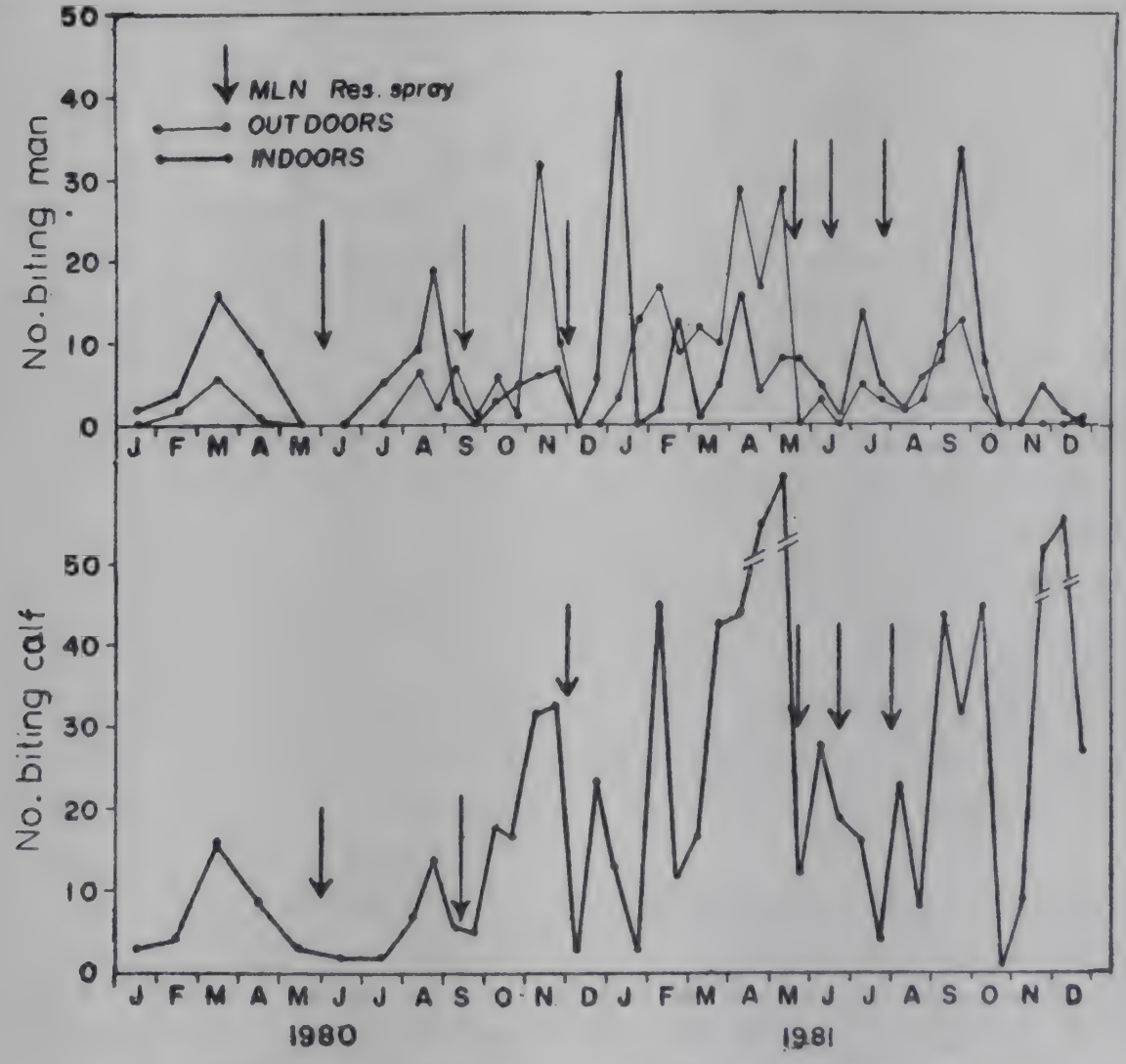

Fig. Biting density *A. culicifacies* of on human and calf baits.

Table III. Analysis of ovarian condition of *A. culicifacies* females captured on bait

Parity status	Christopher's stage					
	1	2a	2b	3	4	5
Nulliparous	37	54	13	2	—	—
Parous	0	95	86	20	—	—
Not determined	0	2	2	1	35	94
Total	37	151	101	23	35	94

blood meal, and pre-gravid nulliparous females taking their second blood meal with ovaries from stage 2a-3 were also represented in the catches.

Daily survival rates : Gillies[4] has pointed out that in species where younger mosquitoes tend to rest outdoors, collections from indoor resting places will be biased in favour of older females. Accordingly only females captured at bait or recently fed were taken into consideration for the calculation of daily survival. Since in Pakistan[5] as well as in Sri Lanka[6], mark-release recapture experiments have shown that in nature the gonotrophic cycle takes between 2 and 3 days, the value of n was taken to be 2·5, following the Sri Lanka study[7]. The daily survival rate was 0·71 to 0·76 in the months February-April 1981. In May and June it was 0·81 and 0·83 respectively, while from July to January 1982 it was continuously high, varying from 0·88 to 0·92.

Vectorial capacity : When calculating the vectorial capacity (n), the duration of the extrinsic incubation period, was taken to be 8 days for *Plasmodium vivax* and 9 days for *P. falciparum* on the basis of temperature[2], except during May and June, when values of 7 and 8 respectively were selected. Following Molineaux and Gramiccia[3], age determinations made during the same season were pooled in order to give more reliable estimates based on larger numbers of dissections. Thus, from February to April the probability of survival through a single day (p) was 0·74 (138 specimens) in May-June $p=0·82$ (365 determinations) and July to January $p=0·89$ (1205 determinations). Similarly, it has been shown that there is a period of higher anthropophily from February to July[8]. The combined anthropophilic index for this period was 0·2567 (1494 determinations), while for the period August to January it was 0·0395 (2685 determinations). The average period between bloodmeals was taken to be 2 days, since some females feed the day after the bloodmeal, and others after the completion of the gonotrophic cycle of 2-3 days (again following the logic of Molineaux and Gramiccia[3]). The average number of *A. culicifacies* biting a man in a night was based on all night biting collections. The values obtained were highly variable, partly due to control measures, as shown in the Fig.

The vectorial capacities obtained ranged from 0-1·079 for *P. vivax* in 1980, and from 0·0499 to 2·9140 in 1981. (Table IV). Corresponding figures for *P. falciparum* are 0-0·9607 in 1980 and 0·0444-2·5937 in 1981. Seasonal trends are not clearly apparent due to the fluctuations in biting density brought about by residual spraying. Vectorial capacities were relatively high in 1981 all through the year as compared to 1980.

In the present study *A. culicifacies* was found to be biting man freely both inside and outside human dwellings. During the hot summer months the inhabitants of the riverine villages slept almost entirely outdoors, but large numbers of freshly fed females, many of them man-fed, would be found in the morning in houses which had been empty at night. Thus, exophagy may be followed by endophily, as has been reported to occur in Afghanistan[9], Sri Lanka[10] and Pakistan[11]. Another finding of interest was that almost 30 per cent of the total catch at bait was composed of females which had already fed once. Refeeding during the same gonotrophic cycle by about 7 per cent of females has been reported,[12,13] but the proportion in this area appears to be much higher. However, collections at bait do not necessarily mean that all the females would have fed, and further investigation is warranted since a higher frequency of refeeding would further increase the vectorial capacity of the local vector population.

Macdonald[14] had calculated from original data of Russell and Rao[15] that the daily survival rate of *A. culicifacies* at Pattukottai was 0·775. In Pakistan, estimates using Davidson's method varied from 0·669 in November to 0·849 in September[16]. In Sri Lanka, values between 0·74 and 0·88 were recorded[7] while immediately after residual spraying with malathion daily survival fell to 0·44. The survival rates for Sri Lanka are closest to the values obtained in the Thenpennai riverine tract. The relatively higher survival may be associated with the high humidities recorded close to the river[17]. By contrast, in the relatively hot and dry conditions of Rameswaram island, lower values ranging from 0·51 to 0·73 have been obtained, using the same method[18].

Few estimates of vectorial capacity are as yet available for *A. culicifacies*. In Pakistan values of $<1\cdot01\times10^{-2}$ for *P. vivax* and $<6\cdot43\times10^{-3}$ for *P. falciparum* were obtained[11], and it was suggested that these extremely low values represent an unstable malaria situation in a non-epidemic year. In Rameswaram island, the values ranged from 0·029 to 0·331 for *P. vivax* and 0·017 to 0·239 for *P. falciparum*. The high values obtained in the present study are due to the high vector density throughout the year, together with the relatively high anthropophilic index in the hot months and the high survival rate during the wet and cool months. Potentially, the most dangerous period is in the middle of the year, when both man feeding and survival are high. However, in both years of the study mass radical treatment was carried in the study villages during this period, which may have prevented outbreaks at this time, and in both years, peak malaria incidence was observed in March-April and in November[17]. However, the area is still highly receptive, and if the percentage of gametocyte carriers in the population (which is at present only 0·7%[17])

Table IV. Vectorial capacity of *A. culicifacies* at the village Kolamanjanur

Month	ma		a	p	n	Vectorial capacity (C)			
						P. vivax		*P. falciparum*	
	1980	1981				1980	1981	1980	1981
January	1·0	14·75	0·0197	0·89	8, 9	0·0665	0·9809	0·0592	0·8737
February	3·0	10·25	0·1278	0·74	8, 9	0·1146	0·3916	0·046	0·2893
March	11·0	7·0	0·1278	0·74	8, 9	0·4202	0·2674	0·3105	0·1976
April	4·5	17·75	0·1278	0·74	8, 9	0·1719	0·6781	0·1270	0·5010
May	0	11·25	0·1278	0·82	7, 8	0·0	1·8056	0·0	1·4808
June	0	2·25	0·1278	0·82	7, 8	0·0	0·3611	0·0	0·2962
July	2·5	6·75	0·1278	0·89	8, 9	1·079	2·9140	0·9607	2·5937
August	9·25	3·25	0·0197	0·89	8, 9	0·6151	0·2161	0·5479	0·1924
September	2·75	15·75	0·0197	0·89	8, 9	0·1829	1·0474	0·1628	0·9324
October	3·0	2·75	0·0197	0·89	8, 9	0·1995	0·1829	0·1776	0·1628
November	13·75	1·25	0·0197	0·89	8, 9	0·9144	0·0831	0·8140	0·0740
December	1·5	0·75	0·0197	0·89	8, 9	0·0998	0·0499	0·0888	0·0444

ma=average no. bites/man/night; a=average no. of feeds taken on man/mosquito/day; p=probability of survival through one day; n=duration of the extrinsic incubation period for *P. vivax* and *P. falciparum*; C=vectorial capacity $ma^2p^n\text{-}\log_e p$. Approximate dates of malathion residual spraying are underlined.

were to be permitted to increase due to any relaxation of surveillance or radical treatment, explosive outbreaks could be expected to take place.

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Density of *Anopheles culicifacies* Giles in relation to control measures along the river Thenpennai (Tamil Nadu)

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Although *A. culicifacies* was susceptible to malathion in the riverine villages of Thenpennai, relatively high vector densities could be detected after residual spraying in unsprayed structures and in unsprayed niches in sprayed structures. The proportion of parous females in the population remained unchanged as a result of incomplete spray coverage. Further, the extended transmission season made it impossible to give effective coverage with three rounds of spraying. It is recommended that the present vector control measures could be supplemented by strengthening antilarval measures, and by peri-domestic ground applications of ULV cold aerosol using technical malathion.

It has been shown that relatively large numbers of the malaria vector *Anopheles culicifacies* can be found resting indoors and biting in villages along the river Thenpennai, which show a high incidence of malaria, even though the area is under indoor residual spraying with malathion[1,2]. Since the vector was susceptible to the insecticide, an analysis was undertaken to identify the causes for the continuing high vector densities, in order to devise an alternate strategy of control.

Material & Methods

Mosquitoes were captured on a timed basis (15 min/structure) using suction tube and torch. Outdoor resting mosquitoes were captured from natural resting places and pit-shelters. All-night biting captures were made on human and cattle bait; and in order to increase the sample for dissection freshly-fed females resting on walls during the night were also collected. Captured mosquitoes were identified, sexed and the physiological age of females were determined by a combination of Detinova's and Polodovova's techniques[3].

Susceptibility tests were carried out by the standard WHO methods. Contact bioassays were carried out on various sprayed surfaces using the plastic containers designed at the VCRC, Pondicherry (Annual Report of the VCRC, 1979). Ten to fifteen fully fed A. *culicifacies* from a laboratory colony of the local strain were used per container and exposed to the treated surfaces for 1 h.

Control measures and surveillance for malaria cases were carried out by the Tamil Nadu Public Health Service; from July 1981 onwards, additional fever surveillance in the indicator villages was carried out by the staff of the VCRC. Cases of malaria detected were reported to NMEP, who undertook radical treatment.

Intervention measures by the Tamil Nadu Public Health Service : The villages in which these studies were carried out *viz.*, Sathanur Dam, Kolamanjanur, Edathanur and Agarampallipattu, received 3 rounds of residual spraying with malathion (target dose 2 g/m^2) annually. These rounds were carried out in May-June, September, and November-December, 1980, and April-June, June-July and July-August 1981. Agarampallipattu received a special round of spray in November 1981. Dates of spraying in each village are shown in the Fig. Recorded coverage of rooms ranged from 39 to 86 per cent. Two rounds of mass radical treatment were carried out each year during the spraying season. The drug regimen for adults was day 1—600 mg chloroquine and 30 mg primaquine, day 2—30 mg primaquine, day 3—15 mg primaquine. This is a modification of the 5 day treatment recommended[4] by the NMEP, which is at present recommended by the State Directorate for the radical treatment of *Plasmodium vivax*. Between 14·3 and 81·8 per cent of the population of each village took the treatment for all three days. In addition a few rounds of cold aerosol treatment were carried out, as indicated in the Fig. and antilarval measures were in progress.

Results

Susceptibility and contact bioassays : Susceptibility tests carried out on wild caught *A. culicifacies* in the village of Kolamanjanur in July 1982, showed that the vector population was susceptible, complete mortality being obtained with half an hour's exposure to the diagnostic concentration of malathion.

Two series of contact bioassays were carried out after routine spraying with malathion WDP in the same village. Mortalities of over 50 per cent were obtained for about four weeks on mud walls and palmyra leaf thatch and for up to nine weeks on grass thatch (Table I).

Vector population before and after spray : The per-man-hour indoor resting density, in relation to spraying for each of the indicator villages, is shown in the Fig. There was usually some degree of reduction in resting density in the month after spraying more marked in 1981, when the rounds were timed one month apart for effectiveness, in view of the bioassay results reported here. Since complete spray coverage was never obtained, vectors could be found resting both in unsprayed rooms and unsprayed niches in sprayed rooms (Table II). Palmyra leaf thatch in particular provided 'refugia', where mosquitoes could be collected 24 h after spraying. Being multilayered, the leaflets in front protected those behind from the spray. Per-man-hour density in unsprayed rooms was higher than in sprayed rooms, but relatively higher numbers could be collected in the latter in 1981 when compared with 1980.

On careful analysis no clearcut increase was found in outdoor resting density immediately after spraying in any of the villages. All-night biting collections on bait in the village Kolamanjanur, made between 1 and 10 days before and

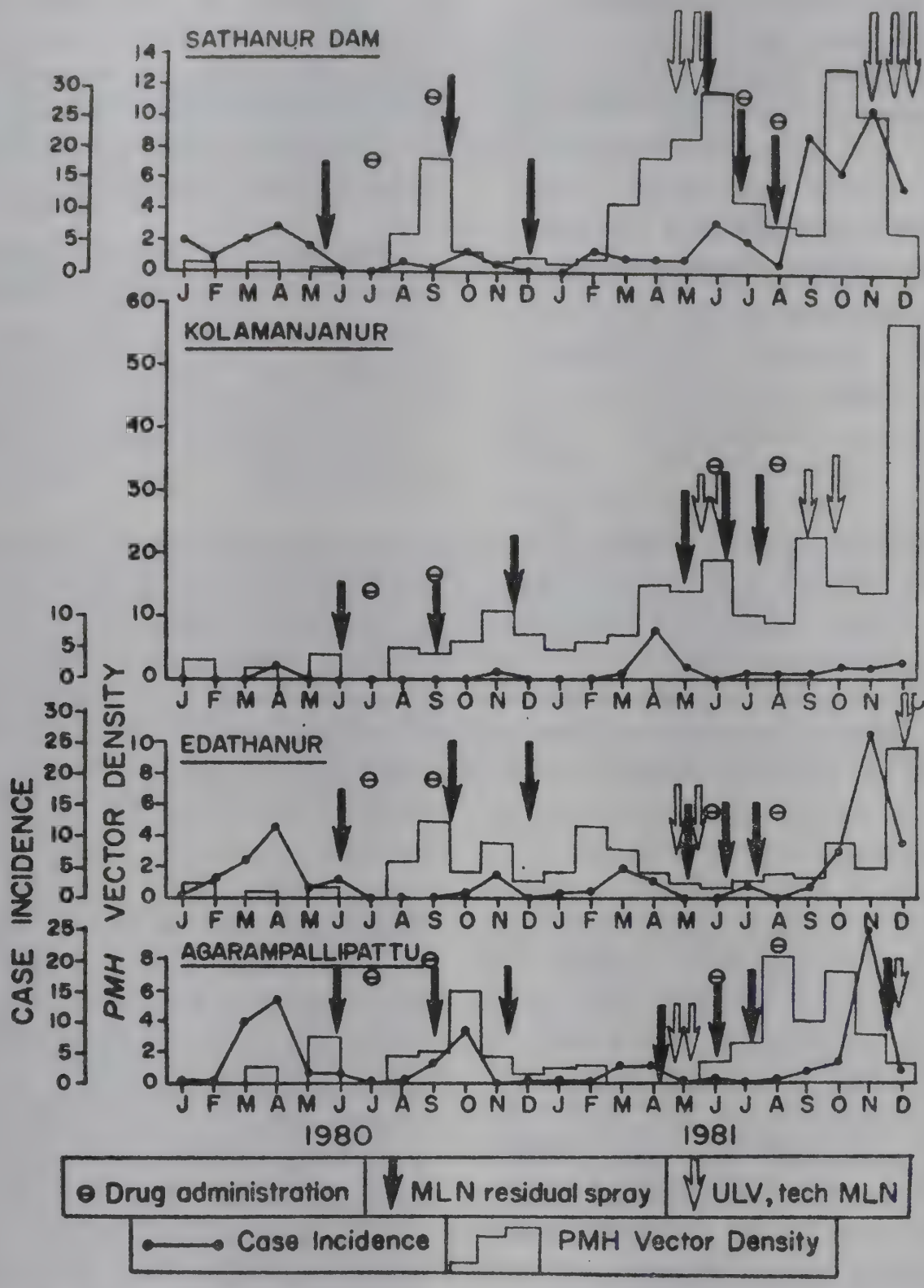

Fig. Indoor resting vector density and case incidence in relation to intervention measures in four villages.

after spray, showed that some reduction in biting activity was usually achieved, except after the first round of 1980, when population density was minimal even before spraying, and the last round of 1981, when an increase in the numbers biting calf bait was observed (Table III).

Age composition of the population : The age composition of catches of *A. culicifacies* before and after spraying were compared during 1981. No reduction in the parous rate was detected after spraying (Table IV). Females with up to four dilatations in the ovary could be found

Table I. Bioassays of malathion residual spray (2g/m²) on various surfaces in a village of North Arcot district, using colony reared *A. culicifacies*

Surface	Spraying date	No. of days after spary										% mortality					
		8-10	17	23-25	30-32	37	42	49-52	60	67							
Mud wall	I	1-12-80	87·2	44·4	70·0	0·0*	—	—	25·9	33·3	25·0						
	II	10-2-81	80·0	—	55·5	0·0	0·0	10·0	—	—							
Palmyra thatch	I	1-12-80	81·6	33·3	—	30·6	—	0·0	44·4	0·25							
	II	10-2-81	100·0	—	60·0	40·0	33·3	30·0	50·0	—							
Grass thatch	I	1-12-80	100·0	88·9	71·4	100·0	88·8	—	87·5	66·7	50·0						
	II	10-2-81	100·0	—	94·1	10·0	—	20·0	60·0	—	—						
Mud wall (control)	I	—	22·0	10·0	0·0	10·0	—	—	10·0	10·0	20·0						
	II	—	10·0	—	5·0	10·0	10·0	11·1	—	—	—						

*Wall had been replastered. Experiment continued in a fresh spot

Table II. Captures of *A. culicifacies* in sprayed and unsprayed rooms in the month after residual spraying

Year	Spray round	No. per man-hour (no. man hours)	
		Unsprayed rooms	Sprayed rooms
1979	III	5·67 (0·50)	0·0 (15·00)
1980	I	3·13 (48·25)	0·13 (24·00)
	II	4·37 (23·25)	1·21 (43·50)
	III	4·27 (14·75)	1·39 (33·50)
1981	I	7·16 (62·25)	4·01 (95·25)
	II	5·51 (57·75)	1·71 (76·00)
	III	5·56 (66·50)	3·13 (92·75)

resting indoors after spray, and females with up to five dilatations were captured biting both before and after spray. The overall parous rate actually increased after the first two rounds of spraying, probably due to the effect of season on adult longevity.

Epidemiological effect of intervention : The collective effect of control measures has to be considered, since mass radical treatment was carried out simultaneously with residual spraying, and in 1981, two cold aerosol treatments at about the same time as the first spray round. In both years of the study there were peaks in malaria incidence in March-April and October-November. These peaks occurred in the individual villages (Fig.) as well as in the whole Primary Health Centre[5]. No control measures, apart from some antilarval work, are normally undertaken between January and the end of April. The highest

Table III. All-night captures of *A. culicifacies* on bait before and after residual spraying with malathion at Kolamanjanur

Spray round	Time period	No. captured on					
		Man indoors		Man outdoors		Calf open shed	
	Year	1980	1981	1980	1981	1980	1981
I	Pre-spray	0	8	0	29	3	86
	Post-spray	0	8	0	0	2	12
II	Pre-spray	3	5	7	3	6	33
	Post-spray	0	1	1	0	5	19
III	Pre-spray	7	5	10	3	33	4
	Post-spray	0	2	0	2	3	23
Total	Pre-spray	10	18	17	35	42	123
	Post-spray	0	11	1	2	10	54

Table IV. Age composition of catches of female *A. culicifacies* before and after residual spraying with malathion in 1981

		Total dissected	No. of dilatations						Proportion porous
			0	1	2	3	4	5	
Indoor resting catch :									
Pre-spray	I	162*	—	—	—	—	—	—	0·47*
Post-spray	I	284**	—	—	—	—	—	—	0·73**
Pre-spray	II	31	13	16	2	0	0	0	0·58
Post-spray unsprayed houses	II	120	39	66	11	2	2	0	0·68
Post-spray sprayed houses	II	99	25	64	10	0	0	0	0·74
Pre-spray	III	127	32	83	9	3	0	0	0·75
Post-spray unsprayed houses	III	106	36	64	5	1	0	0	0·66
Post-spray sprayed houses	III	86	31	45	10	0	0	0	0·63
Night biting catch :									
Pre-spray	I	157	67	82	6	1	0	1	0·57
Post-spray	I	99	38	46	12	3	0	0	0·62
Pre-spray	II	40	22	13	3	2	0	0	0·45
Post-spray	II	70	15	37	11	6	0	1	0·79
Pre-spray	III	112	33	66	11	2	0	0	0·71
Post-spray	III	158	46	97	13	2	0	0	0·71

*76 and **284 specimens were parous on the basis of tracheolar coiling method. Number of dilatations not determined

incidence in three of the four villages during the study period took place in November 1981, in the absence of control measures, which had been completed by August 1981. After this outbreak, a special round of spraying was carried out in one village and ULV cold aerosol treatment in all four.

In general, therefore, malaria incidence was lower during the period of control measures, although the small peak in incidence which occurred during the spraying season in October-November 1980 shows that these were not completely effective.

Discussion

After 15 rounds of residual spraying since 1977, *A. culicifacies* in the Thenpennai riverine tract is still susceptible to malathion. While some reduction was observed after each spray round in both indoor resting and biting densities, it was disturbing to find that there was no reduction in the parous rate in either type of collection. The situation appears to have deteriorated since October 1978, when Kalra (unpublished data) during a tour to this area found few *A. culicifacies* in houses after spraying (0·8 per-man-hour) of which only 0·25 were parous. In the present study between 0·63 and 0·74 of the females found resting indoors after spraying, were parous. As pointed out by Hamon *et al*[6], when parous rates of biting populations of anophelines are unaffected by indoor residual spraying this may be due to inadequate spray coverage or to exophily. While exophily is certainly contributing to the difficulties of achieving complete interruption of transmission, it is clear that the major factor here is inadequate coverage, since high densities of vectors, some with up to four dilatations in the overiole, could be captured indoors immediately after spraying.

Two operational problems are involved, the first being a high proportion of refusals to permit spraying. Lack of public cooperation is a recognised problem in the National Programme of Control, which is becoming steadily more acute as the villagers become more and more unwilling to put up with repeated inconvenience without noticeable relief from the disease. Even when spraying is carried out, walls are frequently replastered with cowdung, and palmyra leaves in particular provide many unsprayable niches. The second problem is the extended transmission season in this area, transmission being possible throughout the year due to high vectorial capacity[2,5]. It is well known that malathion on surfaces like mud walls is relatively short-lived[7], and it is therefore impossible to give an effective coverage throughout the season with three spray rounds a year. Spacing the spray rounds at monthly intervals for maximum effectiveness, as was attempted in 1981, left the last part of the year with virtually no control measures, and was followed by increased malaria transmission.

The vector control measures in operation require supplementation. Antilarval operations in the riverine tract need to be strengthened. However, because of the extensive breeding and inaccessability of many breeding habitats[5], this by itself, may not be sufficient. Peri-domestic ULV cold aerosol treatment has several advantages as a supplement to indoor residual spraying. Thus it would be acceptable to the villagers since it would not involve entering the houses, and the mist would

penetrate into niches inaccessible to conventional sprays. Moreover, outdoor resting mosquitoes which had entered the village for feeding after dark would be killed by the treatment. The treatment should be carried out systematically to cover the period between spray rounds with particular attention to the periods February-March and September-October, in order to prevent the peaks of malaria incidence that have occurred at these times during recent years.

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Epidemiological aspects of malaria in Rameswaram island (Tamil Nadu)

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Rameswaram island, situated in Palk Bay on the south east coast of India, has been endemic for malaria caused by both *Plasmodium vivax* and *P. falciparum*. During the years 1977-1982, the API ranged from 56·40 (1982) to 516·85 (1979) and SPR from 9·73 (1982) to 40·02 per cent (1979) with ABER from 28·23 (1978) to 129·15 per cent (1979). Transmission occurs throughout the year, despite vigorous larvicidal, imagicidal and chemotherapeutic measures carried out by the State NMEP. The problem of persistent transmission is analysed and the epidemiological aspects of malaria discussed in this communication.

Malaria caused by both *Plasmodium vivax* and *falciparum* has been persistent in Rameswaram island despite vigorous control measures carried out by the State National Malaria Eradication Programme (NMEP). The Vector Control Research Centre (VCRC) of the ICMR established a Field Station (March 1981) to make an indepth study of all aspects of the problem of persistent malaria. We describe here the salient ecological features of the island conducive for persistent transmission and epidemiological aspects of malaria analysed from the NMEP data.

Study area : Rameswaram Island (09° 16′ N latitude, 79° 18′ E longitude) is situated at the western extremity of Adam's Bridge, extending east and west between Sri Lanka and the south-eastern coast of India (Fig. 1). It is separated from the mainland by the Pamban channel and has an area of about 55 sq km with a population of about 55,600 (1981 census).

The island has a sub-tropical climate, with mean maximum temperature ranging from 34·4°C in hotter months (April-May) and 29·0°C in cooler months (November-January). The relative humidity ranges from 68 per cent (February-March) to 80 per cent (November-December) with an average of 73 per cent. The average rainfall is 992·9 mm (average for fifty years), fed by both south-west (April-June) and north-east (October-December) monsoons, the latterc ontributing about 75 per cent of the total rainfall.

The terrain of the island is irregular with sand dunes and depressions which lead to creation of vast water bodies during the monsoon. The sandy soil supports the natural growth of thorny *Accacia* sp., *Pandana* sp. and *Borossus flabellifer*. Coconut plantations abound in the eastern, central and western parts. Casuarina is grown by the Tamil Nadu Forest Department in the southern part of the island.

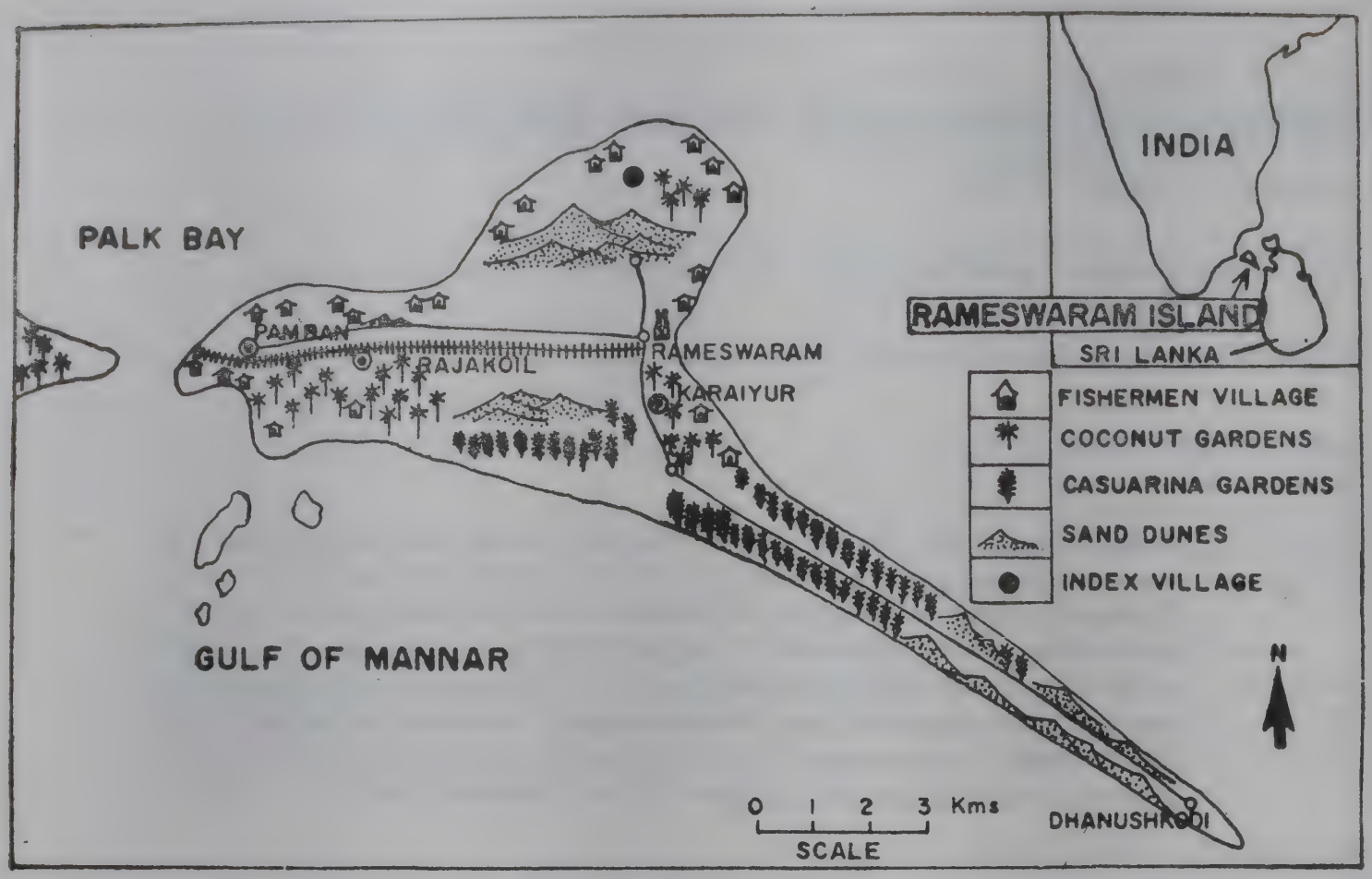

Fig. 1. Map showing study area.

Innumerable pits are dug inside the coconut and casuarina plantations to water them. According to the State NMEP enumeration, there are about 6675 pits and 4672 wells in the island.

The population is distributed in one big town *i.e.*, Rameswaram, two small towns *viz.*, Pamban and Thangachimadam, and 55 villages. Most of the villages are located in the coastline of the island and majority of the people are fishermen living in thatched huts. However, in Rameswaram, Thangachimadam and Pamban, there are also many brick/concrete houses. The Island being a pilgrim centre has a large floating population from all parts of India. The Island is also a visiting point for people travelling to and from Sri Lanka which is only 23 km away.

While the exact census of cattle population is not available their population is relatively scarce in the island when compared with mainland and therefore cattle-sheds are also very rare in the villages. The few cattle present are allowed to move freely and they wander away from the vicinity of the human habitations.

History of malaria : Rameswaram island presents a classical picture of man-made, unstable, coastal malaria. The disease has been endemic since several decades. The earliest published record of malaria in the island was in 1929 when parasitological and spleen surveys were done in 16 localities[1]. The parasite rate ranged from 6 to 72 per cent in different villages and the spleen rate ranged from 3 to 82 per cent. The vector in the island was supposed

to be *Anopheles culicifacies* as it was in the coastal mainland. No other record is available on the malaria prevalence in the island for the periods before the area came under the NMEP except the records on the total number of cases being 1546 to 3629 during 1931-1935 (Viswanathan, D.K. unpublished report). The island came under the NMCP in 1953 and under the NMEP in 1958. It appears that malaria ceased to be a problem in the island during 1958-1967, but from 1968 onwards sporadic cases were recorded (2 in 1968, 11 in 1969, 9 in 1970, 21 in 1971 and 16 in 1972). In 1973 malaria reappeared in epidemic proportions. The number of positive cases recorded in different years were : 663 in 1973, 3605 in 1974, 1321 in 1975 and 2628 in 1976. It has been reported that *P. falciparum* was the predominant species prior to 1974 and that in 1975 nearly 80 per cent of the cases were due to *P. falciparum* and the remaining due to *P. vivax*.

In 1974, massive control operations involving the introductin of larvivorus fishes such as *Poecilia* and *Gambusia* in pits and wells, application of ML oil and Paris green (1%) as larvicides together with residual spraying with DDT (4 rounds) as imagicide were instituted. Residual spraying and weekly application of Paris green were continued till the end of 1977 (three rounds in 1975 and two rounds each in 1976 and 1977). However, the number of cases started increasing steadily from 1976 onwards (Table).

In 1978, the State NMEP switched over to three rounds of HCH (10%) spraying, but the antilarval measures were totally withdrawn. Following this, the highest number of cases reaching a total of 28,427 was recorded in 1979 (API : 516·85) and hence two rounds of malathion fogging and larviciding with Temephos were resorted to, in addition to one round of HCH spraying.

Though there was a decline in malaria incidence in 1980 (there was also corresponding reduction in ABER), the API remained as high as 174·01 while SPR 34·60. Markedly, the SFR recorded was the highest in 1980 (5·25). Again there was a change over to malathion residual spraying (25% WDP-3 rounds) and reintroduction of larviciding with Paris green in 1980. From 1981 onwards, due to poor coverage as a result of refusal from public, residual spraying was replaced by ULV application of malathion (Technical grade). Six rounds of ULV spray in 1981 and 12 rounds in 1982 were carried out. Further decline in incidence was observed during 1981-1982. However, an API of 75·97 and 56·40 and SPR of 19·11 and 9·70 were recorded in 1981 and 1982 respectively. The percentage of *P. falciparum* cases steadily increased from 0·72 to 27·65 during 1977-1982.

Pattern of incidence : While cases were recorded from all over the island, the villages along the southern coastline of the island associated with dense coconut/ casuarina gardens had contributed larger number of cases because of the abundance of breeding places. Cases of both *vivax* and *falciparum* malaria occur throughout the year. Both sexes of all age group were affected, indicating an active transmission.

Analysis of the data on monthly incidence of malaria during the years 1977 to 1982 shows that the peak incidence occurs between January-March (Fig. 2). The peak coincides with the post-monsoon (post-rainy) season when there is heavy vector breeding. Incidence of malaria has been very high with wide variations between

Table. Malaria incidence* in Rameswaram island from 1977 to 1982

Year	Total blood smears examined	No. positive	No. (%) of			ABER	API	SPR	SFR	Total rainfall
			P. vivax	*P. falciparum*	Mixed					
1977	31442	5838	5793 (99·23)	42 (0·72)	3 (0·05)	57·16	106·0	18·57	0·13	1510·5
1978	15526	5848	5742 (98·19)	89 (1·52)	17 (0·29)	28·23	106·32	37·67	0·57	1111·9
1979	71032	28427	26083 (91·75)	2102 (7·40)	242 (0·85)	129·15	516·85	40·02	2·96	1223·9
1980	27664	9571	7973 (83·30)	1451 (15·16)	147 (1·54)	50·3	174·01	34·60	5·25	932·5
1981	22106	4224	3632 (85·98)	560 (13·26)	32 (0·76)	39·76	75·97	19·11	2·53	671·6
1982	32231	3136	2232 (71·17)	867 (27·65)	37 (1·18)	57·97	56·40	9·73	2·69	978·6

*Data obtained from State NMEP

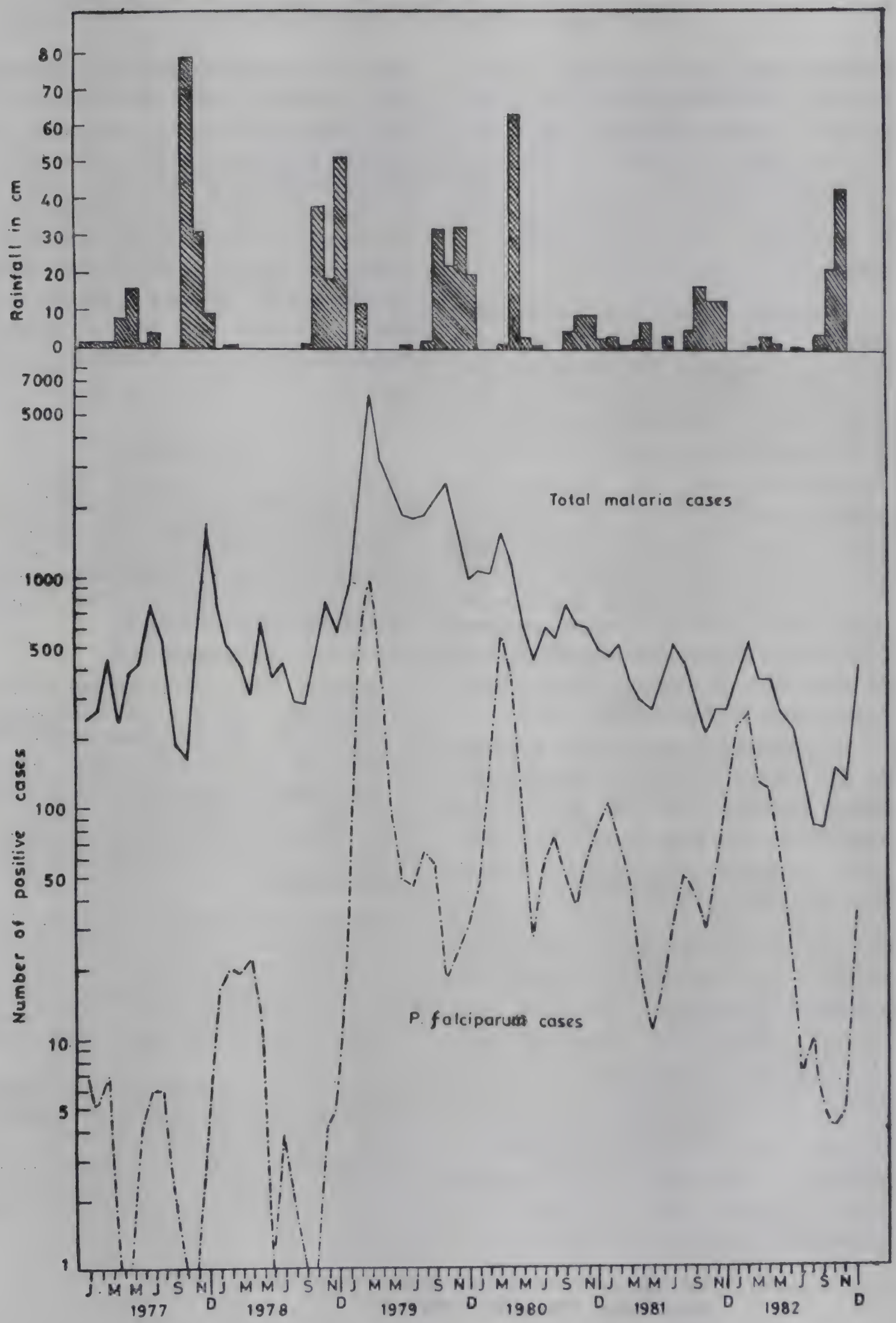

Fig. 2. Incidence of malaria in Rameswaram island from 1977-82.

different years and the variation corresponded to the fluctuations in the annual rainfall. A drastic reduction of total rainfall as a result of drought conditions in 1980 and 1981 had certainly caused drying up of many breeding habitats, resulting in a decrease in the annual incidence of malaria.

Though *P. vivax* is the predominant species, *P. falciparum* cases were also recorded throughout the year except during the months of April, 1977 and June and October of 1978. The peak incidence of *P. falciparum* cases was also found during January-April, the post-monsoon period, when a relatively cooler climate prevails (Fig. 2). This is due to the increased breeding potential following heavy rainfall with consequent build-up of vector density and relatively a higher longevity of the vector in the cooler climate. It is to be noted that in January 1982, *P. falciparum* cases outnumbered *P. vivax*.

No published information is available on any studies on vectors in Rameswaram island between 1958 and 1977. It was reported in 1978 that out of 91 *A. culicifacies* dissected, one was found positive for heavy gland infection[2]. However, many hypotheses were propounded to explain the high level of transmission of malaria despite control operations. *A. varuna* was suspected to maintain a low grade transmission during the offseasons, while *A. culicifacies* was to cause intense transmission during the post-monsoon period (Kalra, N., unpublished report, 1978). Another view was that *A. varuna* has replaced *A. culicifacies* in Rameswaram island (Ganesan, A.V., personal communication). However, there is no published evidence based on any systemaitc study available to substantiate these views. The situation therefore warranted an extensive and indepth study on the ecology and behaviour of the vector(s) involved, its resistance status against different insecticides, on the epidemiological and socio-economical aspects of the disease which are essential for devising a suitable and effective strategy for malaria control. Hence the VCRC undertook studies on all aspects and the findings are presented in this volume[3,4].

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Natural infection & vectorial capacity of *Anopheles culicifacies* Giles in Rameswaram island (Tamil Nadu)

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The role of different species of anophelines in the transmission of malaria in Rameswaram island (Tamil Nadu), where malaria is persistent, was studied. A total of 12,177 specimens, belonging to 7 species, obtained from different types of collections in three index villages over a period of 21 months, were dissected and natural infection was found only in *Anopheles culicifacies* indicating that this is the only vector species involved. The vectorial capacity of *A. culicifacies* was calculated for different seasons from parameters obtained during the study.

Anopheles culicifacies Giles is the major vector of malaria in the plains of Indian sub-continent. The prevalence of this species in Rameswaram island, where malaria has been persistent in spite of control operations[1] was reported as early as 1936 (D.K. Viswanathan, unpublished report on anti-malarial operations) and a sporozoite positive specimen was found[2] in 1978. However, there are other species like *A. varuna* and *A. subpictus* present in the island. While the former has been considered as a vector elsewhere[3], the salt water forms of the latter has been known to transmit malaria in Java[4] and recently in Pudukuppam[5]. In view of the high prevalence of malaria in the island, it was long suspected that other anopheline species may also play a role in the transmission. Therefore, a systematic study was carried out to determine the role of different species of anophelines in malaria transmission.

Material & Methods

Extensive entomological studies were carried out in three selected study villages *viz.*, Pamban, Rajakoil and Karaiyur, which had consistently reported large number of malaria cases[6]. All the anopheline species captured in different types of collections were dissected and examined for natural infection of malaria parasites and age graded following the method of Polovodova[7]. Full-fed specimens of *A. culicifacies* from indoor resting collections were used for sampling blood smears for

precipitin tests and hence they were not examined for gut infection. The vectorial capacity ('C') for *A. culicifacies* was calculated for different months using Macdonald's formula as modified by Garrett-Jones[8] :—

$$C = \frac{ma^2p^n}{-\log_e p}, \text{ where}$$

ma^2 is the number of *A. culicifacies* collected biting a man per night, averaged from both outdoors and indoors in different index villages and multiplied by human blood index obtained from precipitin tests and the reciprocal of the gonotrophic cycle; p is the probability of daily survival calculated from the proportion parous with a gonotrophic cycle assumed to be constant at 2 days, throughout the year.

Since both *Plasmodium vivax* and *P. falciparum* positives were found throughout the year, the value of n (extrinsic incubation period) was assumed to be 7 days from March to October and 8 days from November to February for *P. vivax*; n was assumed to be 8 and 9 days respectively for these two periods for *P. falciparum*, based on temperature conditions.

Results & Discussion

A total of 12,177 specimens belonging to 7 species of anophelines collected during the study period were dissected for oocysts and sporozoites. The different species (with the number dissected given in parenthesis) were : *A. culicifacies* (4371), *A. subpictus* (7391), *A. varuna* (202), *A. hyrcanus* gr. (77), *A. pallidus* (68), *A. tessellatus* (48) and *A. vagus* (20). Only *A. culicifacies* was found positive for sporozoites (21 specimens) and oocysts (3 specimens). None of the other anopheline species dissected were positive for infection, indicating that *A. culicifacies* is the only vector species transmitting malaria in the island. The infected specimens were obtained from all types of collections *viz.*, 17 sporozoite and 2 oocyst positives from indoor resting catches, 3 sporozoite positives from outdoor human biting and 1 sporozoite and 1 oocyst positives from indoor human biting collections. On pooling data, sporozoite positives were found in all the months except May and August; failure to find positive specimens in these two months may be due to chance. The data showed that there was perennial transmission, and since infected specimens were found in all the index villages, transmission was widespread. Both indoor and outdoor transmission was also indicated by the presence of gland positive specimens in biting collections indoors and outdoors. The number of oocyst positives found may be lower than actuals since the full-fed specimens were not examined for gut infection.

The data on survival rate, vectorial capacity, sporozoite rates, number of malaria cases in index villages and the meteorological data are presented in the Table. The probability of daily survival ranged from 0·42 to 0·75 in different months, the highest being between October and March, when the maximum temperature was lower. The vectorial capacity ('C') varied between 0·002 and 0·268 for *P. vivax* and between 0·001 and 0·202 for *P. falciparum*. It varied with the survival rate in different months. Though no distinct correlation was observed between the number of *P. vivax* cases and vectorial capacity, the value was found higher in the months when more *P. falciparum* cases were recorded. The extrinsic incubation period was longer for *P. falciparum* than for *P. vivax* and hence *P.*

Table. Estimated vectorial capacity, probability of daily survival of *A. culicifacies*, sporozoite rates, number of malaria cases and meterological data in Rameswaram island

Month	Temp. °C	Rain fall in mm	Probability of daily survival	Vectorial capacity		Sporozoite rate	No. of malaria cases	
				P. vivax	*P. falciparum*		*P. vivax*	*P. falciparum*
May 1981	34·4	71·2	0·56	0·074	0·043	0	109	4
June	32·7	0·4	0·57	0·048	0·028	1·95	127	12
July	33·4	32·1	0·42	0·002	0·001	0·43	170	17
August	32·8	0	0·69	0·174	0·119	0	149	16
September	32·4	53·3	0·61	0·079	0·048	1·69	87	10
October	32·0	170·2	0·75	0·268	0·202	1·00	74	9
November	30·0	138·2	0·69	0·087	0·059	2·47	114	25
December	28·7	141·1	0·73	0·121	0·088	0·82	68	62
January 1982	29·0	0	0·63	0·014	0·009	0	53	62
February	30·3	0	0·73	0·203	0·148	0·79	108	68
March	32·5	6·2	0·66	0·156	0·102	0·67	75	59
April	34·6	31·3	0·63	0·143	0·091	1·26	106	62
May	34·2	15·5	0·68	0·123	0·085	0	78	42
June	33·8	0	0·44	0·003	0·001	0	87	13
July	33·5	1·5	0·52	0·005	0·003	0	46	6
August	33·9	1·0	0·57	0·012	0·007	0	23	1
September	33·9	42·7	0·62	0·018	0·011	0	22	5
October	32·5	221·5	0·54	0·015	0·008	0·80	63	2
November	29·9	447·6	0·55	0·011	0·007	0	43	2
December	28·3	211·3	0·52	0·013	0·008	0·31	133	17
January 1983	28·9	10·0	0·49	0·004	0·001	0	141	8

falciparum cases were more when the survival was relatively higher.

Between October 1982 and January 1983, both resting and biting densities were high[6]. However, the low vectorial capacity observed in these months was due to the low survival rate of the vector. The number of cases due to *P. falciparum* during these months was also lower, when compared with the previous year. When there is heavy breeding in rainy season, there are more nulliparous mosquitoes in the biting population and hence a lower survival rate may be observed, as has been found in another study[9]. North-east monsoon failed during the season between October 1981 to January 1982, resulting in a decrease in daily emergence and consequently more parous mosquitoes were present in the biting population. The vectorial capacity thus remained high during this period. There was not much change in the daily temperatures during the rainy seasons in the two years. The figure of probability of daily survival obtained was assumed to be constant for the month and this parameter was not obtained every day. Hence there is an approximation in this estimation since many factors like the number of days of rainfall, the day of collection, efficiency of antilarval measures and daily recruitment rate influence daily survival rate.

A lower estimate of vectorial capacity for *A. culicifacies* ($<1{\cdot}01\times10^{-2}$ for *P. vivax* and $<6{\cdot}43\times10^{-3}$ for *P. falciparum*) has been reported in Punjab, Pakistan, during a non-epidemic year[10]. The estimated vectorial capacity for the same species in Rameswaram was relatively higher, which may be attributed to the high anthropophilic index of this species in this area. However, the data show that transmission occurred even when the vectorial capacity was very low (0·001). In a study in Sudan Savanna[11], it was found that even a drop in the vectorial capacity below the critical level need not necessarily prevent transmission when provided with large reservoir of parasites. Uninterrupted transmission occurred in Rameswaram even when the vectorial capacity was low, due to the high parasite reservoir in the human population.

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Density & biting behaviour of *Anopheles culicifacies* Giles in Rameswaram island (Tamil Nadu)

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The resting and biting behaviour of the malaria vector, *A. culicifacies* in Rameshwaram islands, was studied from May 1981 to January 1983. *A. culicifacies* was found to be mainly endophilic. The indoor resting density ranged between 0·36 and 6·99 per man hour in different months. The man biting rate (number biting per man per night) was high in the hot months March-May and ranged between 0·75 and 7·93. The man-to-cattle ratio in the island was 73 : 1. The human blood index for *A. culicifacies* was 0·63. The vector mosquito showed distinct preference to human blood when cattle blood was also offered simultaneously.

Density and biting behaviour of *Anopheles* constitute important aspects of malaria epidemiology. The intensity of malaria transmission in a community depends on the degree of man vector contact[1], and the efficacy of imagicidal measures in a control programme depends on the resting behaviour of the vector species. *Anopheles culicifacies* has been incriminated as the only vector[2] in Rameswaram island, (Tamil Nadu) where malaria is persistent[3]. Studies on the resting and biting behaviour and density pattern of this species in Rameswaram were therefore carried out from May 1981 to January 1983.

Study area : The topography, climate, vegetation and malaria situation in Rameswaram island has been described earlier[3]. Three villages were selected for carrying out extensive studies, as they contributed about 60 per cent of all malaria cases in the island. The village Pamban is located on the western tip of island having a population of about 5000. Rajakoil is about 4.5 km east of Pamban with a population of 850. Karaiyur is adjoining Rameswaram town on the eastern coast having a population of about 2000. There are very few cattle sheds in these villages as the majority of the people are fishermen. The villages are surrounded by extensive coconut gardens and the pits inside these gardens are the major sources of vector breeding.

Material & Methods

Mosquito collections were made indoors using aspirator and flashlight, for 10 min in 35 fixed catching stations in each

study village and in possible outdoor resting habitats like bushes, below culverts, tree holes, crevices in coconut garden pits, wells *etc.* A trap was used between 5·30 pm and 7·30 pm to capture any resting mosquito coming out of the wells.

Indoor and outdoor man-biting night collections were made at fortnightly intervals between 18·30 h and 06·30 h, and all mosquitoes probing to bite or biting were collected. The catching stations were selected randomly. There was no bias in the selection of catching stations. Simultaneous indoor and outdoor biting collections using both human and cattle bait at the same site were also made in all the three villages between 6·30 pm and 7·30 pm once a month. In addition, a magoon trap with a calf bait was set up in each of the three index villages. All anophelines were identified and recorded. *A. culicifacies* females were separated, classified according to the stages of abdominal condition and dissected for age grading and parity[4]. Blood smears from full-fed *A. culicifacies* taken in indoor resting collections were sent to the WHO Reference Centre, Silwood Park, UK for precipitin test. Meteorological data were obtained from the 'Observatory Station' Pamban.

All the data were analyzed statistically.

Results & Discussion

A total of 436 indoor resting collections were made and 3716 *A. culicifacies* were collected in 4199 man hours. Other species collected in order of their abundance were *A. subpictus*, *A. varuna*, *A. hyrcanus* gr., *A. pallidus*, *A. tessellatus* and *A. vagus*. In 75 outdoor resting and well trap collections, only 9 *A. culicifacies* were obtained, 8 in well traps and one in a pit, indicating that this species is predominantly endophilic in Rameswaram.

A. culicifacies density and man biting rates in indoor and outdoor collections along with the meteorological data, are shown in the Table. The density ranged from 0·36 pmh to 6·99 pmh showing high densities during November to March. There was significant negative correlation between the resting density and maximum temperature ($P<0·01$). There was near drought conditions in the island following the failure of the north-east monsoon in the year 1981 and the island recorded normal heavy rainfall in the months of October to December 1982 resulting in increased breeding surface area and higher density in this season.

A. culicifacies formed 54·2 per cent of all the man-biting anophelines collected the other species being *A. subpictus* (38%) and small numbers of five other anophelines. A total of 729 *A. culicifacies* were obtained in man-biting collections over a period of 21 months. The number collected per collection was too small to permit any comments on the hourly preference for their biting. While there was significant partial correlation between outdoor biting rates and relative humidity ($P<0·05$), there was no relationship between biting rates and rainfall and temperature.

The man-biting rate was more or less same in both indoor and outdoor man biting collections. The rate was relatively higher in the drier months from May to September of 1981, a similar trend also being observed during March and April of 1982. Higher man biting rates were also observed during December 1982 and

Table. Number of *A. culicifacies* collected per man hour, indoors, and man biting rate (number collected biting one man per night) both indoors and outdoors, along with meteorological data

Month	Rain fall in mm	Temp.°C		Mean RH	Resting density indoor	Man biting rate	
		Max.	Min.			Indoor	Outdoor
1981 :							
May	71·2	34·4	27·9	71	0·58	8·43	4·6
June	0·4	32·7	27·2	73	0·88	7·0	2·17
July	32·1	33·4	26·8	72	1·76	2·92	0·57
August	0	32·8	26·6	73	1·34	4·0	1·50
September	53·3	32·4	26·2	78	1·09	4·17	2·0
October	170·2	32·0	25·9	78	0·67	1·67	2·0
November	138·2	30·0	25·1	80	0·61	1·67	2·5
December	141·1	28·7	23·7	80	0·75	1·33	1·83
1982 :							
January	0	29·0	24·0	72	1·61	0·50	1·17
February	0	30·3	27·0	69	2·01	1·83	3·33
March	6·2	32·5	25·0	68	1·98	2·50	5·17
April	31·3	34·6	27·3	70	1·47	3·5	7·17
May	15·5	34·2	28·2	72	1·27	1·5	3·0
June	0	33·8	27·9	73	0·83	2·33	2·33
July	1·5	33·5	27·0	74	0·36	0·33	1·83
August	1·0	33·9	27·0	72	0·43	1·0	1·17
September	42·7	33·9	26·8	73	0·51	0·83	0·67
October	221·5	32·5	25·7	76	0·83	2·33	2·33
November	447·6	29·9	24·9	83	3·09	2·33	2·67
December	211·3	28·3	24·6	79	6·99	6·33	4·17
1983 :							
January	10·0	28·9	24·1	75	2·86	1·67	4·0

January 1983, which may be attributed to increased breeding following heavy north east monsoon rains. Rameshwaram island is under intensive larval control measures using Paris green carried out by the State NMEP, which will have to be taken into account while studying the seasonal trend. Though malaria transmission occurred throughout the year and large number of malaria positive cases are recorded every month[3], the routine larvicidal measures were intensified during the post-monsoon months in an attempt to cut down transmission. It is of significance that even when there was low density, transmission of malaria continued unabated.

It has been reported[5] that the per man hour critical density of *A. culicifacies* may correspond to an average of 2·5 bites per man per night. The data in the present study show that the man biting rate was higher than the observed critical density for *A. culicifacies* in several months, both indoors and outdoors, which generally corresponded to the months of high malaria prevalence[3]. The

finding of both oocyst and sporozoite positives in indoor and outdoor biting collections in the study area[2] show that transmission occurred both indoors and outdoors.

The abdominal condition of 3829 *A. culicifacies* females from resting collections were as follows : unfed (517), full fed (1492), semi-gravid (1146) and gravid (674). The difference in distribution of females with full fed and semi-gravid conditions was not statistically significant ($P>0{\cdot}05$). The finding of large number of semi-gravids in indoor resting collections has been reported[1] to indicate the endophilic nature of the species, which has also been substantiated by the finding of very few mosquitoes in actual outdoor resting collections.

A. culicifacies obtained in biting collections were age graded. Of the 601 females dissected, 387 (64·4%) were nulliparous, 193 (32·1%) had one dilatation and 21 (3·5%) had two dilatations. The preponderance of nulliparous mosquitoes in biting collections suggests the proximity of breeding places. In an area subjected to intensive antilarval measures, this would indicate a failure of the control measures.

Preference for human blood was exhibited by *A. culicifacies* in Rameswaram. In a total of 537 blood smears, 330 or 62·9 per cent reacted with human antisera. This may be attributed to man cattle ratio being 73 : 1. High anthropophilic index was also observed in Ennore[6] having scarcity of cattle. However, in 69 simultaneous cattle and man-biting collections made in Rameswaram in different seasons, 22 *A. culicifacies* were taken biting man, as against only 5 specimens from cattle. In magoon traps with calf bait set up along with all-night man biting collections, 277 *A. culicifacies* were collected biting man, whereas only 66 specimens were obtained in the magoon traps. There is, thus an overwhelming evidence that the strain of *A. culicifacies* found in Rameswaram, exhibits a distinct preference for human blood. This high preference for human blood exhibited by *A. culicifacies* is one of the major contributing factors for high transmission of malaria in the island, the other factors being a high gametocyte load in the community[2] and perennial breeding of the vector species.

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Persistent malaria transmission in Pudukuppam— a coastal village of Pondicherry

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Pudukuppam, a coastal village inhabited by fishermen has been highly endemic for malaria for many years. Extensive studies were carried out to incriminate the vector involved in the persistent transmission of malaria in this village. *Anopheles subpictus*, an ubiquitous mosquito, was found to be the predominant species breeding and its role in the local transmission of malaria was demonstrated. Natural infection in wild mosquitoes was obtained during the period, April to August when both mosquito density and malaria incidence were at a peak.

Malaria has been persisting in some coastal villages of Pondicherry despite the control measures taken by the State National Malaria Eradication Programme (NMEP). The problems of the coastal villages are unique and complex in nature and containment of malaria in such situations poses a difficult task. One such village from which high incidence of malaria has been reported every year is Pudukuppam, situated about 18 km south of Pondicherry. This village is inhabited by fishermen who make frequent visits, while fishing, to other areas on the Coromandel coast extending from Rameswaram in south to Visakapattinam in north. The fishermen acquire infection from some of these places and form, on return, the foci of local transmission. Due to the free availability of chloroquine and other antimalarial drugs, most of them escape detection and radical treatment. Therefore, the gametocyte load in the community has always been moderate to heavy. *Anopheles subpictus* was found to be the most predominant mosquito in this region and this species has been incriminated as a vector of malaria recently[1]. This mosquito has been reported to be an active vector of malaria in Maldive islands[2] and in some other areas of South East Asia[3,4]. Extensive studies were carried out to understand the complexity of the problems involved in the persistence of malaria in this coastal village and the results are presented.

Study area : Pudukuppam village has a population of about 800 constituted mostly by fishermen living in thatched huts. The men and children sleep outdoors except in the rainy season. There are no cattle domesticated in this fishing village. The mosquitogenic potential of this village is also very high due to the abundance of a variety of breeding habitats. There is a

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large area of backwaters adjacent to the village which is an outspread of the river Mooliar. The village is surrounded by *Casuarina equisetifolia* plantations, a commercial crop raised in the sandy coastal belt. Innummerable pits are dug to water these plants which form one of the important breeding places of mosquitoes. The rainy season starts in May and extends up to December and the total rainfall in 1981 was 3193 mm spread over 48 days.

Material & Methods

Active case detection was carried out and both thick and thin smears collected from all fever cases, stained with JSB and examined. All persons with fever were given presumptive treatment. Those who proved positive for malaria parasite were later questioned about their movements during the preceding 45 days, duration of stay outside the village, any history of previous attack, treatment taken *etc*. The positive cases were classified as indigenous, relapse or imported and their family records were maintained. Positive cases were later given radical treatment. Mass blood surveys were carried out during the month of May 1981 and April 1982. Data on the malaria incidence for the years 1978 and 1979 were obtained from State NMEP, while data for 1980, 1981 and 1982 were collected by the Vector Control Research Centre (VCRC) itself.

Systematic mosquito collections were carried out during study period of April 1981 to September 1982, employing standard entomological techniques. All night human biting collections and indoor resting collections were carried out once a week from April 1981. For man-biting collections, urchins from the village itself were selected and the same baits volunteered for the entire duration of study. The volunteers, with minimum clothing, were allowed to sleep and all the mosquitoes biting or attempting to bite were collected. The collectors were rotated in a shift system. Indoor resting collections were made at weekly intervals from fixed stations. All the mosquitoes collected were brought to the laboratory, held in cloth cages in a temperature controlled room ($28°\pm2°C$), identified and dissected for examining the gut and salivary gland infections.

The vectorial capacity was calculated using Macdonald's formula modified by Garett Jones[5].

Results & Discussion

According to the records of NMEP, there were 33 and 72 positive cases in 1978 and 1979 respectively in a population of 800. Cases were detected by the VCRC for the years 1980, 1981 and 1982 and 150, 220 and 169 cases respectively were detected using an efficient door to door surveillance system. Out of 1250 fever cases detected over the study period of 18 months, 330 were found positive for *Plasmodium vivax*. While blood smears from all age groups and both sexes were positive for malaria, it is significant to note that 7·8 per cent of the 330 positive smears were from infants below one year. Of the rest, 49 (14·8%) were from age group 1 to 5 and 60 (18·1%) from children between 6 and 10 yr. The positivity rate in the active surveillance was 26·4 per cent; 22 patients (6·6%) gave a history of having gone out of the village prior to acquiring infection, 166 (50·3%) had not gone anywhere outside the village, 97 (29·3%) were confirmed relapses and 45 (13-6%) were those

who either refused to give information or were not available for questioning. The incidence of malaria in children below 10 yr who generally do not move outside and formed one third of all cases, suggests indigenous transmission.

In the mass blood survey, 610 and 622 blood smears respectively were collected and examined in 1981 and 1982 and 35 (5·7%) and 16 (2·5%) slides were positive for *P. vivax*. Of these, 21 were from asymptomatic carriers. The species composition and the relative density of mosquitoes collected resting in human dewellings are presented in Table I.

A. subpictus was found to be the most predominant mosquito species attacking man. The outdoor sleeping habits of the villagers appear to facilitate a better man-vector contact for exophagy in this species. Of the 9372 anophelines (*A. subpictus*, *A. vagus*, *A. pallidus* and *A. nigerrimus* gr.,) collected on human baits, 9339 (99·6%) were *A. subpictus*.

A total of 8417 *A. subpictus* females were dissected and 59 were found with gut infection and 4 with gland infection. The positives were found during the months April to August in both the years, when there were generally more number of malaria cases (Table II). Though cases

Table I. Per man hour density of indoor resting mosquitoes in Pudukuppam

Month	Rainfall (mm)	*A. subpictus*	*C. sitiens*	*C. vishnui*	*C. tritae-niorhynchus*
1981					
April	0	5·85	0	2·00	0
May	189·9	12·50	0·75	3·41	0·41
June	4·1	6·57	1·65	0·37	0·02
July	188·6	19·41	7·75	1·31	1·60
August	125·8	16·50	1·50	1·35	0·21
September	114·8	18·63	0·09	0·45	0
October	360·8	11·18	0	0·18	0
November	120·0	32·68	0	0·50	0·25
December	195·6	4·77	0	0·22	0
1982					
January	0	14·13	0	0·30	0
February	0	24·40	0·06	0	0
March	0	35·31	0	0	0
April	0	1·50	0	0·16	0
May	13·0	5·70	0	0·10	0
June	35·9	6·50	0·16	0	0
July	85·0	15·16	2·16	1·83	0·16
August	28·0	15·44	0·85	0·66	0
September	67·0	34·70	0	0	0

Small numbers of *C. bitaeniorhynchus*, *C. whitmorei*, *Armigeres subalbatus*, *Mansonia uniformis* and *M. annulifera* were also collected in some months

Table II. Natural infection of *A. subpictus* with human malarial parasite, manbiting rate* and incidence of malaria in Pudukuppam

Month	No. of mosquitoes dissected	No. infected		Man biting rate	No. of malaria cases
		Gut	Gland		
1981					
April	383	9	0	39·6	33
May	1554	16	0	79·9	4
June	1160	14	0	43·6	43
July	1177	9	2	132·2	44
August	963	4	2	191·8	22
September	455	0	0	69·3	6
October	155	0	0	43·5	11
November	32	0	0	74·5	9
December	254	0	0	44·0	7
1982					
January	77	0	0	60·8	8
February	452	0	0	59·4	17
March	270	0	0	84·5	28
April	45	0	0	18·0	17
May	181	3	0	30·5	26
June	291	2	0	31·5	20
July	217	2	0	114·5	7
August	353	0	0	39·5	17
September	398	0	0	6·5	11

*Number biting one man per night

were detected in all months of the year, there is no clear evidence to indicate that all the cases detected were of indigenous origin because of frequent movement of the fishermen to adjoining malarious areas.

Significantly, the man biting rate of *A. subpictus* was high throughout the year. The gut and gland positive *A. subpictus* were found in the months when relatively more number of malaria cases had occurred in this village. The probability of daily survival was calculated for different months (Table III) and was found to be high in all months with an increase during the months April to August. This period also corresponds to a higher vectorial capacity and occurrence of more number of malaria cases when compared to other months. Undoubtedly, *A. subpictus* showed a high vector potential for transmitting malaria in Pudukuppam area, especially in the absence of other recognised malaria vectors and this may be the situation in similar areas elsewhere. The high values of vectorial capacity for *A. subpictus* could be due to higher anthropophilic

Table III. Estimated vectorial capacity (C) of *A. subpictus* along with monthly rainfall, man biting rate and number of malarial cases in Pudukuppam

Month	Rain fall mm	No. of malaria cases	Man biting rate	Survival rate P	Vectorial capacity C
1981					
April	0	33	39·6	0·92	11·50
May	189·9	4	79·9	0·93	29·08
June	4·1	43	43·6	0·89	6·95
July	188·6	44	132·2	0·93	48·11
August	125·8	22	191·8	0·92	55·72
September	114·8	6	69·3	0·87	7·71
October	360·8	11	43·5	0·89	6·93
November	120·0	9	74·5	0·88	9·89
December	195·6	7	44·0	0·89	7·05
1982					
January	0	8	60·8	0·80	2·16
February	0	17	59·4	0·80	2·11
March	0	28	84·5	0·80	2·99

Vectorial capacity, $C = \frac{ma^2p^n}{-\log_e p}$ and ma^2 is the product of man-biting rate multiplied by human blood-index (0·145) as determined by precipitin tests and the reciprocal of gonotrophic cycle (0·33), *P* is the probability of daily survival calculated from parous rate and n is the extrinsic incubation period (8 days)

index exhibited by this species in Pudukuppam which has no cattle, high man biting rate and survival rates. Vectorial capacity decreased drastically from August onwards as a result of intensive vector control operations, as also observed in the Garki Project[6]. During the rains and post-monsoon months, there were more nulliparous mosquitoes in man biting collections which was reflected also in the relative decrease in survival rate.

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Laboratory studies on the biology of *Anopheles subpictus* Grassi, 1899

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The biology of *A. subpictus*, which has been incriminated as a vector of malaria in Pondicherry recently, was studied in the laboratory. A cyclic colony was raised for this purpose. The fecundity ranged from 38 to 286. The mean hatchability of the eggs was 64·7 per cent. Highest insemination rate was observed when the male to female ratio was 5 : 1. The longevity of females ranged from 2-21 days.

Anopheles subpictus is widely distributed in the oriental region[1] and has been incriminated recently as a vector of malaria in coastal villages of south east India[2]. This species was also reported to be a vector in Maldive islands[3], Indonesia[4] and in the south coast of Java[5]. Literature available on the biology of this mosquito is meagre, as this species was not amenable to colonization. A cyclic colony of this species has been established in the laboratory at Vector Control Research Centre (VCRC), Pondicherry[6]. The results of studies on some aspects of biology of this species are presented here.

Material & Methods

The material was obtained from the cyclic colony maintained at this Centre, and all the studies were carried out under controlled conditions (28±2°C, RH 60-75%). Fecundity was determined by counting the number of eggs laid by individual females confined in vials with water for oviposition. To determine the hatchability, larval survival, pupation rate and sex ratio of emerging adults, the eggs obtained from individual females were reared to adult stage in enamel trays (48×14×5 cm) containing tap water provided with standard larval diet and the trays exposed to sunlight for 5 to 6 h daily.

The effect of desiccation on egg viability was studied by keeping known numbers of freshly laid eggs on moist and dry mud for varying duration and later floating them in water. Immature duration was studied at different densities ranging from 1000 to 25000 m^2 water surface. The effect of interspecific competition among the larvae of *A. subpictus*, *Culex quinquefasciatus* and *C. tritaeniorhynchus*, which breed together in nature in some habitats was studied by rearing known number of larvae of each species together in different larval rearing media such as tap water, water from ground pool, backwater and coir-pit and the number of

adults emerged in each species was recorded. The effect of blood meal and sex ratio on insemination rate was studied by keeping fed females with males at different sex-ratios in one cubic foot cloth cages with simulated twilight conditions. The longevity and gonotrophic cycle were determined in January (winter), May (summer), August (autumn) and November (monsoon), signifying the different seasons by keeping freshly emerged adults in a colony cage (60 × 60 × 60 cm) providing raisins and fowl for blood meal daily until all die. The number of eggs laid in ovitraps every day were counted and plotted. The peaks in the number of eggs laid were considered as the days of gonotrophic cycle.

Results & Discussion

The number of eggs laid by a single female in the first oviposition cycle averaged 194±7?, with a range of 38 to 286. The mean hatchability was 64·7 per cent. The rate of pupation ranged from 15·8 to 80·7 per cent with an average of 50·2 per cent. The females slightly outnumbered the males and the average male to female sex-ratio of 13 cohorts at emergence was 1 : 1·04. The eggs remained viable for a longer duration on moist soil, than on dry soil. Resistance to desiccation of eggs was also observed in the case of *A. albimanus*[7].

The mean range of duration of various immature stages studied individually are as follows (h) : eggs, 40·8±0·75; I instar, 33±1·02; II instar, 35·5±2·23; III instar, 43·6±1·65; IV instar, 77·9±3·96; pupae, 27·3±1·32. The duration of the immature stage increased with increase in the density (Table). The impact of overcrowding in immature duration was more pronounced in third and fourth instar larvae and also in pupae. In adult emergence also, a significant decrease was observed as the density increased.

A. subpictus dominated over *C. quinquefasciatus* and *C. tritaeniorhynchus* when reared together in media such as water from ground pool, back water and coir pit, but in tap water medium with artificial diet *C. quinquefasciatus* dominated.

A. subpictus readily took blood meal on

Table. Duration of immature stages of *A. subpictus* expressed by 50 per cent moulting day (with day of initial and last appearence in parenthesis) at different density levels

No. of larvae	Larval density/ m^2	Larval duration				Pupae	% adult emergence
		I	II	III	IV		
96	1000	3 (1-4)	4 (3-5)	5 (4-6)	6 (5-8)	8 (6-9)	89·0
200	3100	3 (1-3)	5 (3-7)	6 (4-8)	7 (6-9)	10 (7-10)	85·0
400	6200	3 (1-3)	5 (2-6)	6 (4-7)	7 (5-9)	9 (6-9)	74·0
500	7760	3 (1-4)	6 (3-7)	8 (6-9)	9 (7-12)	10 (8-12)	73·0
1000	15500	4 (1-5)	6 (3-7)	8 (5-11)	11 (6-14)	13 (8-15)	62·0
1600	25000	4 (1-5)	6 (3-10)	8 (6-11)	15 (7-17)	17 (9-18)	19·0

the day of emergence. Insemination was not found to be a pre-requisite for taking blood in *A. subpictus*. Only on the 5th day after blood meal were the females found inseminated. The male and the female held in ratios ranging from 1 : 1 to 1 : 5 were tested for insemination rate, no female was found inseminated even after 72 h. However, when the male to female ratio was 3 : 1, 30 per cent of the females were found inseminated, and when the ratio was increased to 5 : 1, 50 per cent of the females were inseminated after 72 h.

First oviposition took place on the 7th day after emergence in all seasons. The second peak of egg laying was on the tenth day in the rainy months January and August. In the hot season (May and June), the second peak was on the 9th day. The majority of mosquitoes followed a cycle of 7-2-2 during hot season (*i.e.*, first oviposition on seventh day after emergence and subsequently in every two days) and 7-3-3 during the rest of the year. Longevity of males was found to be higher than females. Fifty per cent mortality of males occurred when they were eight days old and their longevity ranged from 2 to 24 days, while the corresponding periods for females were 7 and 2-21 days. Studies carried out by earlier workers[8] on the longevity of this mosquito in the laboratory showed that they lived for a shorter duration (range 5-11 days) and 50 per cent mortality in females occurred, when they were 5 days old.

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Field observations on the swarming & the mating behaviour of *Anopheles subpictus* Grassi 1899

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The swarming and mating behaviour of *A. subpictus* was studied in nature. Swarming and mating occurred only at dusk and was influenced by the time of sunset and intensity of light. Males always outnumbered the females in all swarms. Both parous and blood fed females participated in swarming. Mating occurred while flying in swarms.

Anopheles subpictus is an ubiquitous species and is widely distributed throughout India. This species has been recently incriminated as a vector of malaria in some coastal villages of southeast India[1]. Isolations of Chittoor, Arakonam and Japanese encephalitis viruses have also been made from *A. subpictus*[2,3]. Literature available on the swarming and mating behaviour of this species is very meagre. Hence a study was undertaken on this aspect.

Material & Methods

Observations of the swarming of *A. subpictus* were made in Nainarmandapam village (situated about 3 km away from Pondicherry town) during dusk hours, an hour prior to sunset until the complete disappearance of swarming, and at dawn. The site chosen for this study was a small lane in the interior of the village. The major indoor resting places of this species determined previously, consisted of a row of eight huts and big cattle sheds on one side and a huge concrete building on the other. The lane opened into a coconut plantation, surrounded by paddy fields which supported heavy breeding of *A. subpictus*.

Observations were made during February, March, April and July. The mean daily temperature ranged from about 25° to 30°C. Winds were moderately strong throughout the year except during February, prior to summer. During the study period, a westerly component predominated in the mornings, while in the afternoons, winds reverted to South easterly in direction. Standard cloth sweepnets were used to collect swarming mosquitoes[4]. Mosquitoes collected in each swarm were brought to the laboratory to determine the species composition, sex ratio, and physiological conditions of females. All the female mosquitoes were dissected and examined the same night for the presence or absence of sperms. The number of mating pairs was also noted and record-

ed. The time of initiation of swarming, duration, direction, the height from the ground level at which swarming occurred *etc.* were also recorded.

Results & Discussion

The time of sunset varied from 1814 to 1850 h on different days (Table). During February, which is a relatively cold month, the time of initiation of swarming ranged from 1 to 23 min after sunset and lasted for 10 to 34 min. In other months, the initiation of swarming varied from the time of sunset to 20 min and lasted for 16 to 21 min. Swarming was usually initiated by a few males and the number increased to thousands with a decrease in the intensity of light resulting in a cloud-like formation with very vigorous movements. The swarm gradually dispersed as darkness set in.

Swarming and mating of *A. subpictus* occurred only during dusk, and no swarming was observed at dawn. At dusk, many mosquitoes were seen flying out of their resting places. Similar observations were also recorded with *Culex quinquefasciatus*[5].

No swarming occurred at the breeding sites or at the ground level. No particular object was found necessary to induce swarming unlike in some other species in which swarming occurred over a stationary marker or moving object[6]. It was also observed that the swarm moved up and down vertically at a stationary place. When a person or cattle walked below, the swarm moved along with the moving objects. *A. subpictus* faced east while swarming. *A. culicifacies* was reported to face east[7] and north-west and occasionally north or west.

Table. Swarming time, sex ratio, abdominal condition, parity and insemination of *A. subpictus*

Date	Time of sunset	Initiation of swarming	min after sunset	Duration of swarming	No. of mosquitoes collected	No. of males	No. of females	Abdominal condition of females				Parity		Insemination	
								Unfed	Fed	Semi-gravid	Gravid	Nulliparous	Parous	Mated	Unmated
15-2-80	18·14	18·15	1	15	267	263	4	4	0	0	0	4	0	0	4
25-2-80	18·17	18·32	15	18	224	178	46	31	14	1	0	32	14	5	41
26-2-80	18·17	18·26	9	34	505	465	40	28	12	0	0	11	29	4	36
27-2-80	18·17	18·40	23	10	104	88	16	7	3	2	4	10	6	2	14
28-2-80	18·17	18·27	10	19	426	401	25	20	3	1	1	19	6	3	22
4-3-80	18·18	18·29	11	16	78	78	0	0	0	0	0	0	0	0	0
14-3-80	18·19	18·39	20	21	85	79	6	6	0	0	0	6	0	1	5
19-3-80	18·20	18·30	10	20	386	382	4	2	0	0	2	ND	ND	1	3
1-4-80	18·20	18·30	10	20	74	72	2	2	0	0	0	1	1	0	2
18-4-80	18·50	18·50	0	20	250	236	14	10	0	4	0	3	11	0	14

ND=Not determined

Males always outnumbered the females. In the samples collected, the male to female ratio ranged from 3.8 : 1 to 95·5 : 1. On one occasion only 78 males and no females were collected. Majority of the females collected were unfed but some fed females were also collected. A few semigravid females were also taken in the swarms. Only 60 per cent of females in the swarms were nulliparous, demonstrating that parous females also participate in the swarming. Participation of older females in swarming activities has been observed among *A. annularis* also[8]. In *A. culicifacies* only nulliparous females have been reported in swarming[9].

Many mating pairs were seen in the swarms but no pair appeared to drop down as observed in *C. quinquefasciatus*[6]. Mating was observed, while flying in swarms. Of the 157 females dissected immediately after swarming, only about 10 per cent were inseminated. This may be because they were perhaps caught before they could copulate.

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Control of *Anopheles subpictus* breeding in backwaters of two coastal villages of Pondicherry

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Backwaters in two coastal villages of Pondicherry, breeding *A. subpictus*, were studied to investigate the role of this habitat in promoting mosquitogenic conditions and to devise suitable methods of control. The relationship between physico-chemical characteristics and survival rate of immature stages and other predatory fauna in the habitat were studied. Extensive growth of algae prevented effective predation of mosquito larvae by the natural enemies present in this habitat.

Some coastal villages with malaria[1] in the Union Territory of Pondicherry have vast expanses of backwaters. These water bodies are highly mosquitogenic and *Anopheles subpictus*, recently incriminated as a vector of malaria in this region[2] breeds in them. Studies were therefore carried out to assess the role of this ecosystem in promoting mosquito breeding with a view to control breeding and the results are presented.

Study area : Backwaters of two coastal villages, Nallavadu and Pudukuppam, situated about 20 km southeast of Pondicherry town were studied. The backwater formation in Nallavadu is due to the blocking of a tributary of the river Chunnambar joining the sea. The water body on the northern side of the village, covering an area of 1·5 km^2 after rains and about 1 km^2 area during the summer, is a permanent one. The waterbody on the southern and south-western sides covering an area of 2·5 km^2 after rains usually dries up from April to September. In Pudukuppam, the backwater formation is due to an outspread of the river Mooliar. This permanent water collection on the southern side of the village occupies about 5·5 km^2 area after rains and about 3·0 km^2 in summer. The backwater region on the north and north-eastern side of the village measuring about 2 km^2, after rains, dries up totally during summer months.

Two species of aquatic algae *viz.*, *Chaetomorpha littorea*, and *Enteromorpha compressa* var. *pinqulata* (fam: Chlorophyceae) grow in this habitat throughout the year. An aquatic grass *Cynodon dactylon* is also found extensively at the edges. Immediately after the rains when the water level increases, the algae are submerged under water, and the algal cover surfaces again in the post-monsoon months.

Material & Methods

Physico-chemical characteristics such as *p*H, dissolved oxygen, salinity, sulphate and nitrate-nitrogen were determined following standard procedures[3]. For estimating the dissolved oxygen, the water sample was fixed at the site by the addition of required volumes of manganous sulphate and alkaline potassium iodide solutions. The surface area of backwaters is too vast and therefore was stratified into five zones in Nallavadu and eight zones in Pudukuppam for sampling purposes to assess mosquito larval density. Twenty dipper samples, ten on the surface of algae and other vegetation and the other ten in the areas free from vegetation were taken about one metre apart in each zone at weekly intervals.

The larvae and pupae collected were brought to the laboratory, counted stage-wise, reared to adults and identified in the laboratory. The survival rates of immatures for different months were calculated following the method of Service[4].

Associated fauna were collected and identified at the Zoological Survey of India, Madras.

Results & Discussion

Vast area of backwaters in the two coastal villages of Pondicherry is one of the most important and perennial source of mosquito breeding. *A. subpictus* is the most predominant mosquito breeding. *A. vagus*, *Culex vishnui* gr. and *C. sitiens* also occur in very small numbers. Extensive growth of aquatic algae on the water surface and grass at the edges of backwaters promote *A. subpictus* breeding. The algal mats not only provide ideal oviposition sites but also shield the mosquito larvae from the predatory fishes and wave action. *C. sitiens* also breeds during the summer months in pits dug for retting coconut husk in the periphery of backwaters.

A. subpictus tolerated high chloride concentrations up to 36,500 ppm which is nearer to that of the sea water (38,500 ppm). The sulphate content ranged from 0 to 1272 ppm in Nallavadu and 0 to 988·8 ppm in Pudukuppam. Nitrogen content in the form of nitrates was found in very small quantities, ranging from 0·04 to 0·18 ppm in Nallavadu and 0·04 to 0·24 ppm in Pudukuppam. Dissolved oxygen was generally high in water collected on algal mats and negligible or absent in the highly polluted water pits where coconut husk is being retted. Earlier studies[5] on anopheline breeding in ditches and ponds had shown that a greater proportion of breeding habitats contained 1 to 4 ppm of dissolved oxygen. The *p*H of backwaters is generally neutral and occasionally shifts towards alkaline range up to 8 and very rarely towards acidic range up to 6.5. Mosquitoes are known to be specific and selective about their breeding places and the physico-chemical factors of the habitat are known to influence the selection of oviposition sites and their subsequent development[6].

The density of immature stages of *A. subpictus* varied in different regions of backwaters and with the season. The deep backwaters of Pudukuppam did not support mosquito breeding during the post-monsoon season, as the algae were submerged in water. Generally, this mosquito is not found in regions devoid of vegetation as a result of continuous wave action.

However, heavy mosquito breeding was observed in small shallow pools on the periphery of backwaters during the same period. During summer months, as the water level receded, algal cover surfaced and this habitat supported mosquito breeding. In Nallavadu, where the backwater is relatively more shallow, rains did not have a pronounced effect on mosquito breeding since the algae were not submerged. Small pools in shallow waters continued to breed until they dried up in summer.

The survival rate of immature *A. subpictus* in backwaters ranged from 0·44 to 11·35 per cent in different months. In Nallavadu where the nature of backwater is different from that of Pudukuppam, survival rate of immatures was high from December to March with a peak in February, the post-monsoon period. In Pudukuppam, a high survival rate was observed only during the summer months (April to July), with a peak in June.

The backwaters generally support a variety of other aquatic organisms. During this study, 22 species of fishes, 3 species of prawns and 4 species of insects were recorded. However, this list is not exhaustive as the study was confined to only the predatory fauna. The fishes recorded were *Etroplus maculatus* (Cichlidae), *E. suratensis* (Cichlidae), *Oryzias melastigma* (Cyprinodontidae), *Gambusia* sp. (Cyprinodontidae), *Aplochellus blochi* (Cyprinodontidae), *Acentrogobius globiceps* (Gobiidae), *Mugil* sp. (Mugilidae), *Macrobrachium* sp. (Palaemonidae), *Therapon* sp. (Theraponidae), *Ambassis gymnocephalus* (Ambassidae), *Hemirhampus limbatus* (Hemirhamphidae), *Gerres limbatus* (Gerridae), *Elops saurus* (Elopidae), *Mystus qulio* (Bagridae), *Nematalosa nasus* (Clupeidae), *Tetrodon oblongus* (Tetrodontidae), *Muraena macura* (Muraenidae), *Rasbora daniconius* (Cyprinidae), *Labeo calbasu* (Cyprinidae), *Puntius sophorae* (Cyprinidae), *Macropodus cupanus* (Belontidae) and *Channa punctata* (Ophiocephalidae). Three species of prawns *viz.*, *Penaeus monodon*, *P. indicus* and *Macrobrachium idea* and four insects species *viz.*, *Hydrophilus indicus*, *Sternolophus rufipes*, *Anisops bouvieri* and *Hydrometra vittata* were also found in the backwater habitat.

Even though many species of fishes, prawns and other predatory insects are present in backwaters, this habitat continued to be an important breeding source for *A. subpictus* as the algal cover shielded mosquito larvae from predation. Mosquito larvae are actually found on the algal cover. Removal of algae is necessary not only to expose the larvae to predation but also to facilitate wave action which is one of the well-known naturalistic methods of larval control. Tonnes of algae were therefore removed manually from backwaters which helped in controlling mosquito breeding over a vast area. But due to continuous growth of algae in backwaters, physical removal had to be a continuous process which entailed considerable labour cost and therefore could not be a permanent solution. The economic utility of algae was explored in the laboratory and it was observed that it could be utilised in hand-made paper industry where waste cotton, tailor cuttings, gunny rags and waste wool are used as raw materials[7]. When algae was used (30-40%) as a substitute to some of these raw materials, it gave a mottling effect to the hand made paper which found a ready export market in addition to the reduction of the cost of

raw material. The dried algae was sold at the rate of Rs. 850 ton as against Rs. 4000 per ton for other waste material. The industrialists were, therefore, very enthusiastic in using algae for paper manufacture. The villagers collected and sold the algae to the hand-made paper industry and this process has helped in finding a permanent solution for mosquito control in this major habitat.

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Salinity tolerance of sibling species in the taxon *Anopheles subpictus* Grassi, 1899

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Two sibling species of the taxon *A. subpictus*, designated A and B and morphologically distinguishable as egg or adult, have been recognised in coastal villages near Pondicherry. In the laboratory, larvae of species A showed high mortality with increasing salinity of the larval medium; total mortality being observed above 2·0 per cent NaCl. This species occurred naturally in habitats with salinity ranging from 0·004 to 0·734 per cent NaCl. Species B was highly saline tolerant, showing less than 16 per cent larval mortality in pure sea water (3·2 % NaCl) in the laboratory and occurring naturally in habitats like back waters of the sea, and coir pits, in which salinity ranged from 0·040 to 5·832 per cent NaCl.

The species *Anopheles subpictus* has long been known for its ability to breed both in fresh and in brackish water. Early workers in India had considered the possibility that there were distinct fresh water and salt water forms and attempts were made to distinguish them morphologically[1]. The theory of 'biological races' of *A. subpictus* had been discarded by 1943 for lack of proof[2]. However, it has recently been demonstrated that there are two sibling species, provisionally designated *A. subpictus* species A and species B, occurring in coastal villages near Pondicherry[3]. Since these could be distinguished from each other as eggs or as adult females[4], it became possible to study their breeding biology, particularly in relation to their salinity tolerance.

Material & Methods

Larvae were collected from natural habitats in coastal localities from Pondicherry to Cuddalore. Concurrently water samples were collected and analysed in the laboratory for chloride and dissolved oxygen content. The larvae were allowed to pupate and adult females emerging were identified by means of the palpal index (*i.e.*, the ratio between palpal segments 5/4). Specimens with indices of 0·55 or less were determined as species A, those of 0·61 and above as species B, and those showing intermediate values were discarded[4].

For testing salinity tolerance wild-caught females were individually tubed and

the egg batches were identified. Pools were formed separately of larvae hatching from A type and B type eggs respectively, and tested either as fourth instar or newly emerged first instar larvae. For each species two replicates of 25 fourth instar larvae were tested at each of 11 concentrations ranging from 1.0 to 3·0 per cent NaCl. The larvae were placed in beakers containing the test solution, and mortality was recorded after 24 h. A similar procedure was followed with first instar larvae in various dilutions of sea water. In this case two replicates of 50 larvae each were tested at each dilution.

Results

Determinations on adults reared from wild collected larvae from habitats of varying salinity are summarised in Table I. It will be seen that species A was found in habitats with salinity ranging from 0·004 to 0·734 per cent NaCl. Only species B was found at NaCl concentrations from 0·967 to 5·832 per cent, and it was not recorded below 0·040 per cent. Both species appeared to be able to withstand pollution, as judged by low dissolved oxygen content (2 ppm and below in casuarina and coir pits, serial nos 2, 5, 11 and 12 in Table I).

In the laboratory fourth instar larvae of species A showed low mortality after 24 h in 1·4 per cent NaCl, and complete mortality at 2·2 per cent and above (Table II). Species B, on the other hand, showed no mortality at concentrations of up to 2·6 per cent NaCl (Table II) and only low mortality (16%) at 3·0 per cent, which is approximately the salinity of sea water.

First instar larvae of species B could survive with only 16 per cent mortality in pure sea water (Table III), while complete mortality of species A was obtained

Table I. Occurrence of sibling species of *A. subpictus* in natural habitats with different salinities

Sl. no.	Date (1981)	Locality	Habitat	NaCl (%)	No. identified	
					Species A	Species B
1.	16·4	Thazanguddai	Casuarina pit	0·004	50	0
2.	16·4	Thazanguddai	Casuarina pit	0·012	24	0
3.	16·4	Thazanguddai	Pool near back water	0·040	41	1
4.	22·1	Pudukuppam	Pool near back water	0·093	0	27
5.	2·3	Nallavadu	Casuarina pit 62	0·734	2	56
6.	15·1	Pudukuppam	Backwater	0·967	0	12
7.	15·1	Backwater	Backwater	2·015	0	27
8.	16·4	Thazanguddai	Backwater	2·178	0	7
9.	2·3	Nallavadu	Casuarina pit 57	2·228	0	25
10.	23·4	Pudukuppam	Backwater	3·836	0	20
11.	23·4	Cuddalore OT	Coir bit	5·131	0	13
12.	23·4	Cuddalore OT	Coir bit	5·832	0	1

at 50 per cent of sea water (equivalent to 1·6% NaCl).

Table II. Effect of sodium chloride concentrations on the 4th instar larvae of sibling species of *A. subpictus*

NaCl %	Mortality %	
	Species A	Species B
1·0	0	0
1·2	0	0
1·4	6	0
1·6	40	0
1·8	64	0
2·0	94	0
2·2	100	0
2·4	100	0
2·6	100	0
2·8	100	14
3·0	100	16

Sea water=3·2%

Table III. Effect of dilutions of sea water on first instar larvae of sibling species of *A. subpictus*

Sea water : fresh water	Mortality %	
	Species A	Species B
10 : 90	0	1
15 : 85	0	2
20 : 80	1	0
25 : 75	3	0
30 : 70	5	0
35 : 65	15	1
40 : 60	31	0
45 : 55	34	0
50 : 50	100	0
75 : 25	100	1
100 : 0	100	16

Discussion

It had earlier been noted that *A. subpictus* species A was found inland as well as in coastal localities, while species B has been found only on the coast[3]. In coastal villages where both occurred, species A—the less saline tolerant species, occupied the fresh water habitats, while the highly tolerant species B was found in brackish habitats up to 5·8 per cent of NaCl. In those parts of South-east Asia where the salinity ranges overlap, *A. indefinitus* occupies the fresh water niche[5] (as in case of *A. subpictus* sp. A, in our study), whereas *A. subpictus* s.l. is mainly confined to brackish water[5]. Breeding has been recorded at a salinity of 8·6 per cent in fish ponds in Java[1], much higher than the values recorded in natural habitats near Pondicherry. The distribution and habitat preferences of the sibling species of *A. subpictus* might be of importance for control if either one of them were to be incriminated as a malaria vector in India.

In Africa, a simple salinity test has been used to discriminate between first instar larvae of freshwater and saltwater species of *A. gambiae* s.l[6]. A similar test could be used to separate the species of *A. subpictus*. Based on the studies reported here a concentration of 3 parts sea water and 1 part fresh water would be a suitable discriminating level for rapid screening.

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Impact of storage period & temperature on the larvicidal activity of bacterial pesticide formulations

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Water-dispersible powder formulations of indigenous strains of *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* H-5 a and b were prepared and stored at —40, 8 and at 30°C and assayed for larvicidal activity against *Culex quinquefasciatus* third instar larvae at weekly interval up to 30 wk. The larvicidal activity of the *B. thuringiensis* H-14 formulation declined slowly upon storage, but the decline was not significant. In the case of *B. sphaericus* formulation, the insecticidal activity deteriorated rapidly at all the storage temperatures to a significant level.

The search for indigenous strains of biological agents for mosquito control has yielded two promising spore forming bacteria, *viz.*, *Bacillus thuringiensis* H-14 and *B. sphaericus* H-5 a and b. Both the agents have been developed into different kinds of formulations by different research organizations and commercial firms. However, detailed studies on their shelf-stability are scanty. Studies were therefore carried out at the Vector Control Research Centre, Pondicherry to assess the stability of the formulations prepared from two indigenous strains of bacterial pathogens.

Material & Methods

B. thuringiensis H-14 (VCRC acc. No. B-17)[1] and *B. sphaericus* H-5 a and b (VCRC acc. No. B-6)[2] were used for the study. Both the bacilli were cultured on nutrient agar medium for two days and their cells harvested and made into water dispersible powder (WDP) formulations. At the time of standardizing the preparation of WDP formulations, it was ascertained that none of the ingredients/additives used in the formulation are deleterious to the very larvicidal property of the two bacterial agents (VCRC Annual Report, 1981). The formulations were dispensed into 10 g samples in sterilized screw capped vials and stored at -40°, 8° and 30°C. The larvicidal activity of the stored WDP formulations was tested against *Culex quinquefasciatus* third instar larvae at weekly interval up to 30 wk. For each treatment, there were 50 larvae per bowl with 250 ml of water in quadruplicate with appropriate controls. Larval mortality was scored 24 h after inoculation, corrected to control mortality, if any, using Abbott's formula[3]. The LC_{50} values

were worked out after transforming the percentage mortality into probit values.

Results & Discussion

The initial potency of *B. thuringiensis* H-14 formulation was equivalent to the LC_{50} dose of 54 μg (Table). After 30 wk of storage at −40, 8 and at 30°C the LC_{50} dose required increased to 80, 76 and 86 μg, respectively. Different storage temperatures equally affected the larvicidal activity of the formulation.

Earlier studies indicate that the *B. thuringiensis* H-14 standard powder, IPS-78, and aqueous suspensions of commercial formulation do not lose insecti-

Table. LC_{50} values of bacterial pesticides for *C. quinquefasciatus* third instar larvae

Storage period (wk)	Storage temperature, °C					
	B. thuringiensis H-14			*B. sphaericus* H-5 ab		
	−40	8	30	−40	8	30
0	54	54	54	500	500	500
1	68	56	62	540	500	520
2	59	55	58	530	540	560
3	63	61	58	570	550	590
4	66	58	64	560	540	580
5	69	62	60	550	575	670
6	68	65	62	560	585	630
7	65	64	65	590	590	640
8	66	68	68	570	610	570
9	65	64	74	610	630	700
10	68	69	66	600	620	700
11	70	72	70	625	640	750
12	74	70	76	680	720	900
13	69	74	73	685	775	940
14	71	76	69	740	820	950
15	65	75	74	720	810	970
16	70	78	68	760	840	1010
17	72	76	72	810	890	1100
18	70	74	73	840	940	1150
19	68	79	76	870	910	1120
20	69	82	74	860	930	1300
21	74	72	78	840	950	1240
22	80	76	75	920	980	1300
23	78	73	80	980	1040	1400
24	82	78	84	1120	970	1400
25	84	81	79	1040	990	1500
26	86	84	82	980	1070	1550
27	82	80	88	1080	1100	1600
28	81	84	86	1120	1140	2000
29	83	80	82	1020	1060	2000
30	80	76	86	1050	1090	2000

cidal activity after prolonged periods of storage *i.e.*, 165 days to 22 months, at room temperature conditions of tropics[4]. However, at 50°C IPS-78 powder showed a slight decrease in its activity. In the present study, the larvicidal property of the *B. thuringiensis* H-14 formulation deteriorated but not rapidly. Compared with the storage period of 30 wk, the loss of larvicidal activity observed was insignificant. Hence it is construed that the *B. thuringiensis* H-14 formulation has better stability irrespective of storage temperatures up to 30 wk of storage.

The initial potency of *B. sphaericus* formulation was equivalent to the LC_{50} value of 500 μg for *C. quinquefasciatus* third instar larvae. Rapid deterioration of its potency occurred as the storage period increased. After 30 wk, the LC_{50} values increased to 1090, 1050 and 2000 μg respectively for the lots stored at −40°, 8° and 30°C (Table).

Earlier studies show that *B. sphaericus* 1593 culture when kept for four months at 21°-22°C, at −20°C and at −80°C and aqueous suspensions of its cells kept at 23°-25°C for about a year did not show noticeable change in the activity[5,6]. But other reports indicate that the larvicidal activity is lost when the culture is exposed to 80°C for 12 min or the cell wall suspension is immersed in boiling water for 10 min[7]. In the present study, the *B. sphaericus* formulation lost its toxicity significantly after 30 wk of storage temperature conditions. The rate of increase in the LC_{50} value of formulation as the storage period increased was multiples of that observed with *B. thuringiensis* H-14 formulation. It is evident therefore, that the *B. sphaericus* formulation has poor shelf-life and thus cannot be stored for longer period.

The study shows that *B. sphaericus* formulation loses insecticidal activity very rapidly on storage, whereas the activity of *B. thuringiensis* H-14 formulation is highly stable. Hence, further efforts need to be made to improve the stability of *B. sphaericus* formulation.

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Evaluation of K-Othrine[R], a synthetic pyrethroid for insecticidal efficacy against mosquito vectors

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The efficacy of K-Othrine[R] (Decamethrin)—a synthetic pyrethroid as larvicide and adulticide was tested against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. The residual effect of the insecticide on different surfaces, *viz.*, cement, mud and thatched, was also determined at dosages of 50 and 100 mg (ai)/m^2.

Chemical control has its own importance in controlling vectors during epidemics even though many older methods like environmental and biological control have greater relevance in the present situation. Studies were carried out on the efficacy of a new synthetic pyrethroid insecticide, K-Othrine[R] (Decamethrin) against vector species at Vector Control Research Centre, Pondicherry, and the results are presented here.

Material & Methods

Decamethrin is a synthetic pyrethroid having a structural formula, (S)-L-cyano-m-phenoxybenzyl (1R, 3R)-3 (2, 2-dibromovinyl)-2, 2-dimethyl-cyclopropane carboxylate obtained from M/S Roussel Pharmaceuticals (India) Ltd., as a 2·5 per cent wdp formulation. Pure decamethrin was crystallised from 5 per cent ethylacetate-petroleum ether as white crystalline needles (melting point=98°-101°C).

Larvae and adults of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* tested were obtained from the cyclic colonies maintained at our Centre. Susceptbility of field collected *C. quinquefasciatus* and a malathion resistant strain of *C. quinquefasciatus* was also tested against decamethrin. Standard WHO procedures[1,2] were followed for determining larval and adult susceptibility tests.

Insecticide impregnated papers of different concentrations were prepared in the laboratory by distributing uniformly acetone solutions of known quantities of decamethrin in olive oil base over Whatman No. 1 filter papers (15×12 cm). The amounts of insecticide and olive oil were adjusted to give the distribution rate of 3·6 mg/cm^2. These papers were used for evaluating the adulticidal efficacy. The standard solutions for larval susceptibility testing were made in ethanol. LC_{50} values were obtained by probit analysis. Control mortality, if any, was corrected

by using Abbots formula[3]. All the experiments were conducted at 25±2°C.

The residual effect of the insecticide on different wall surfaces like cement, mud and thatch was monitored at weekly intervals at the dosages of 50 mg (ai)/m^2 and 100 mg (ai)/m^2 against *C. quinquefasciatus*, *A. stephensi* and *Ae. aegypti* by following a modified procedured developed at the Vector Control Research Centre, Pondicherry (Annual Report of VCRC, 1980).

Results & Discussion

Larval LC_{50} values are presented in Table I. The values show that *C. quinquefasciatus* (lab and malathion resistant strains) was more susceptible to decamethrin than *Ae. aegypti* and *A. stephensi*. The field strain of *C. quinquefasciatus* was found to be about five times less susceptible than the laboratory strain. When the larvicidal efficacy of K-Othrine[R] was compared with the other synthetic pyrethroid insecticide, permethrin[4], it was found that K-Othrine[R] (decamethrin) was four times more potent than permethrin against *C. quinquefasciatus* and *Ae. aegypti*.

The susceptibility values of the adults against decamethrin are given in Table II. *Ae. aegypti* adults were more susceptible to decamethrin followed by *C. quinquefasciatus* and *A. stephensi* as evidenced by the respective LD_{50} values of 0·1128, 0·1420 and 0·6636 $\mu g/cm^2$.

When the adulticidal efficacy of decamethrin was compared with bendiocarb[4] an effective carbamate insecticide, it was found that decamethrin is about 2·5 times more effective against the field strain of *C. quinquefasciatus*, about 6 times more effective against *Ae. aegypti* and about 1·2 times more effective against *A. stephensi*, than bendiocarb.

The residual effect of decamethrin (K-Othrine)[(R)] on different surfaces against the three mosquito vectors are presented in Table III. At 50 mg (ai)/m^2, decamethrin was effective against all the three species on thatched surface for more

Table I. Larvicidal efficacy of K-Othrine[R] against different species of mosquitoes

Species	Regression equation Y=a+bx	LD_{50} (ppm)	95% fiducial limits
A. stephensi	Y=3·72+3·79x	0·0022	0·0021 —0·0023
Ae. aegypti	Y=1·57+3·39x	0·0001	0·00009—0·00011
C. quinquefasciatus (Field)	Y=1·88+1·98x	0·00038	0·00036—0·0004
C. quinquefasciatus (Malathion Res) 75 F 75	Y=2·13+3·74x	0·00006	0·00005—0·00007
C. quinquefasciatus (Lab)	Y=2·18+3·32x	·00007	0·00006—0·00008
C. quinquefasciatus 32 F 75	Y=2·30+2·56x	·00011	0·00008—0·00016

Table II. Adulticidal efficacy of K-Othrine[R] against different mosquito species

Species	Regression equation	LD_{50} $\mu g/cm^2$	95% fiducial limit
A. stephensi (Lab)	Y=1·62+1·86x	0·6636	0·5222—0·8433
Ae. aegypti	Y=2·42+2·45x	0·1128	0·0901—0·1413
C. quinquefasciatus (Field)	Y=2·65+1·80x	0·1990	0·1791—0·2222
C. quinquefasciatus (Malathion Resistant) 75 F 75	Y=3·83+1·03x	0·1376	0·0713—0·2642
C. quinquefasciatus (Lab)	Y=3·17+1·58x	0·1420	0·1018—0·1982

Table III. Residual effect of K-Othrine[R] (decamethrin) on different surfaces against the mosquito vectors

Concentration of the insecticide	Test species	Residual effect (wk)		
		Cement	Mud	Thatched
50 mg (ai)/m^2	*C. quinquefasciatus*	3	3	20
	A. stephensi	Nil	Nil	20
	Ae. aegypti	3	14	20
100 mg (ai)/m^2	*C. quinquefasciatus*	18	19	17
	A. stephensi	5	Nil	17
	Ae. aegypti	10	19	17

than 20 wk. On cement surface, it was effective for 3 wk against *C. quinquefasciatus* and *Ae. aegypti*. On mud surface it was effective for 14 wk against *Ae. aegypti* and for 3 wk against *C. quinquefasciatus*. It was found to have negligible effect against *A. stephensi* on both cement and mud surfaces.

At 100 mg (ai)/m^2, decamethrin was effective for more than 15 wk on mud surface against *C. quinquefasciatus* and *Ae. aegypti* whereas it showed a very little effect against *A. stephensi*. On thatched surface, decamethrin was effective against all the species for more than 20 wk. On cement surface, decamethrin had a residual effect for 18, 5 and 10 wk against *C. quinquefasciatus*, *A. stephensi* and *Ae. aegypti*, respectively.

Application of decamethrin at bimonthly intervals in rural areas where most of the houses have mud walls and thatched roofing can interrupt transmission of malaria.

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Efficacy of Monox CI-FCM in *Culex quinquefasciatus* control in an urban situation

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Monox CI-FCM(R)—a monomolecular layer was tested against *C. quinquefasciatus* in the tanks and pits. Successful inhibition of adult emergence was obtained at a target dose of 1·89 litres per square meter surface area of 1 per cent solution. The effect was more prolonged in the tanks than in the pits.

Three major classes of alternative chemicals are available at present to overcome difficulties faced in vector control programmes due to insecticide resistance. These are synthetic pyrethroids, juvenile hormone mimics and monomolecular surface film. Monomolecular surface films are more acceptable to the environmentalist because they do not lead to any adverse effect on the non-target organism[1-4]. However, this chemical has not yet become operational due to lack of data from large scale field trials to prove its utility. We report here the efficacy of Monox CI against immatures of filariasis vector *Culex quinquefasciatus* in Pondicherry, an urban town.

Material & Methods

Monox CI-FCM (in the form of 15% w/v suspension of insoluble surfactant in water) was supplied by CI Insect Control Ltd., England. Since the monomolecular layers are effective in stagnant water, the efficacy of this compound was assessed in cesspits, cement tanks and septic tanks, which are the preferred breeding habitats of *C. quinquefasciatus* and contain highly polluted water. In these breeding habitats Monox CI-FCM (1% solution) was applied at the rate of 1·89 $1/m^2$ of breeding surface area. Surface application was made with 16 litres capacity Knapsack sprayer with a special nozzle. Pre-spray immature density was noted by dipper sample and post-spray density was evaluated by sampling daily from the treated pits.

Results & Discussion

The trial showed that Monox CI-FCM gives 100 per cent kill of pupae and all larval instars. However, in many pits fresh breeding started from the next day onwards indicating that the stability of the film is not persistent. In cement tanks and septic tanks, the effect was slightly prolonged and the pupae appeared only on 11th day, on an average (Table I).

Table I. Efficacy of Monox cI in the septic tanks and cement tanks

Sl. no.	Surface area (m^2)	One per cent sprayed in litre	No. of replicates	Pupae appeared in days after spray in average
1.*	0·12	0·23	6	7 (6-8)
2.*	0·48	0·91	7	8 (6-10)
3.	0·35	0·66	3	8 (7-10)
4.	0·36	0·68	2	28 (7-48)
5.	0·36	0·68	1	50 (—)
6.	0·49	0·93	5	9 (8-11)
7.	0·66	1·25	6	9 (8-10)
8.	0·80	1·52	6	8 (7-8)
9.	0·96	1·82	5	10 (6-15)
10.	3·6	6·84	3	14 (6-27)

*Septic tanks, others cement tanks

Table II. Efficacy of Monox cI in the transient habitat cesspits

Sl. no.	Surface area (m^2)	One per cent sprayed in litre	No. of replicates	Pupae appeared in days after spray in average
1.	0·2	0·38	1	10 (—)
2.	0·283	0·54	1	12 (—)
3.	0·283	0·54	4	14 (7-28)
4.	0·3	0·57	1	40 (—)
5.	0·385	0·73	6	8 (6-10)
6.	0·385	0·73	4	7 (6-8)
7.	0·42	0·8	6	8 (6-9)
8.	0·48	0·91	1	14 (—)
9.	0·54	1·03	5	7 (7-9)
10.	0·56	1·06	2	17 (8-25)
11.	0·56	1·06	2	17 (6-28)
12.	0·56	1·06	2	12 (7-16)
13.	0·56	1·06	1	6 (—)
14.	0·786	1·49	6	9 (6-13)
15.	0·951	1·81	5	9 (6-14)
16.	1·7	3·23	3	8 (7-9)

In cesspits pupae appeared on an average, on the 10th day (range 6-40 days). Due to regular water flow into these pits from the household, the film was disturbed and oviposition starts at the point of confluence. However, in undisturbed pits the effect was quite prolonged and pupae generally appear on 14 to 15 days (Table II).

Residual effect of this compound in cesspits and septic tanks varied widely depending upon the type of pits and water use. Where large volume of water was used by the householder, the monomolecular film was disturbed and breeding starts. In cesspits and septic tanks it was slightly advantageous in the sense that when the insecticide will kill only up to IV instar larvae, this compound can produce mortality even in the pupal stage thereby preventing emergence. Secondly, in a large scale control programme, large cesspools which are not disturbed can be treated with this compound which gives control for prolonged time, and in some undisturbed pits the breeding was prevented for as long as 50 days.

While the compound cannot be advocated for all kinds of habitats in a control programme, it could be used in some selected breeding places of temporary nature which spring up, after the rainy seasons.

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Field efficacy of *Bacillus thuringiensis* H-14 formulations against mosquito larvae in casuarina & coconut garden pits

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A field trial was carried out with three formulations of *B. thuringiensis* serotype H-14, a microbial pesticide, against anopheline and culicine larval populations in casuarina and coconut garden pits with non-polluted and polluted waters. The formulations tested were Deltox, Bactimos and Teknar at a dose of 1 g, 1 g and 3 ml/10 m^2 water surface respectively. All the formulations were found to be effective against anopheline and culicine larvae in non-polluted as well as polluted waters, causing drastic reduction in larval density within 24 h, after treatment. No larvicidal activity was found beyond 24 h. The minimum lethal dose requirement in the field was found to be higher for Bactimos and Teknar, than for Deltox.

Bacillus thuringiensis serotype H-14 which is advocated as an alternative to chemical larvicides has been found highly toxic to many mosquito species while being safe to non-target organisms and mammals[1]. The efficacy of this agent has also been demonstrated in the laboratory against several vector species and several studies have been carried out in simulated field conditions against mosquito larvae, and in natural habitats[1,2]. A large scale field trial was undertaken using three formulations of *B. thuringiensis* H-14 in natural breeding sites for a period of five weeks, primarily to test the efficacy of an indigenous formulation and also compare its efficacy with two commercial formulations. The results of this field trial are presented in this communication.

Material & Methods

The three *B. thuringiensis* H-14 formulations tested were : (*i*) Deltox, a water dispersible powder prepared at the Central Drug Research Institute (CDRI), Lucknow, from an indigenous strain isolated and developed at the Vector Control Research Centre (VCRC), Pondicherry; (*ii*) Bactimos, a wettable powder of Biochem Product, S.A. Brussel, Belgium; and (*iii*) Teknar, an emulsifiable concentrate of Sandoz Limited, Basle, Switzerland.

The LC_{50}, LC_{90} doses and the potency of the formulations were determined in the laboratory using the Pondicherry strain of *Aedes aegypti* as bioassay

units and IPS 82 standard powder as the reference.

A preliminary field trial was carried out during the dry season in May-June, 1983, in pits used for watering coconut trees in a garden in Rameswaram island where only Deltox was tested. The size of these pits varied with the surface area of water ranging from 3 to 13 m^2 and the height of the water column from 30 to 60 cm. The water in the pits was clear and supported breeding of both anophelines and culicines. Deltox was applied at a dosage of 1 g/10 m^2 in twenty pits, and 2 g/10 m^2 in another 20 pits, and 10 pits were kept as control. The dosage required was arrived at by mixing the formulation in tap water and the formulation was applied uniformly over the water surface by a knapsack sprayer after determining its discharge rate. The immatures were sampled daily before and after treatment by taking 5 dipper samples in each pit, four at the periphery with equal spacing and one at the centre, counted, recorded and later released into the respective pits.

In July 1983, a large scale field trial was carried out in coconut garden pits in Vizhundamavadi village of Vedaranyam, Thanjavur district. Out of 171 pits selected, 130 had no visible organic pollution, whereas water in 41 pits was polluted due to soaking of coconut husks and fronds. The *p*H and temperature of water in pits were measured daily.

Prior to the field trial, tests were carried out in the field to determine the optimum dosage of the three formulations causing 100 per cent mortality of the larvae. The dosage rates tested per 10 m^2 surface area were 1, 1·5 and 2 g of Deltox, 0·5 and 1 g of Bactimos and 1, 2 and 3 ml of Teknar. Since Deltox and Bactimos gave 100 per cent mortality at 1 g each, and Teknar at only 3 ml, these dosages were used in the field trial; 64 of the pits with non-polluted water were treated with Deltox, 8 pits with Bactimos and 18 with Teknar. Of the pits with polluted water, 24 were sprayed with Deltox, 5 with Bactimos and 2 with Teknar. Forty pits with non-polluted water and 10 with polluted water were left unsprayed to serve as control. The method of spraying and sampling has been described earlier. Separate sprayers were used for different formulations. Observations were made for 37 days in the pits treated with Deltox and Bactimos and for 26 days in the pits treated with Teknar. Since pupae appeared in pits treated with all the formulations on the 6th day of post-treatment, reapplication was done every fifth day.

Results & Discussion

The potency of Deltox, Bactimos and Teknar formulations in terms of international toxic units/mg (ITU/mg) were 1373, 8133 and 1721 respectively. The LC_{50} and LC_{90} values against III instar larvae of Pondicherry strain *Ae. aegypti* were as follows : Deltox-71 and 106 mg; Bactimos-12 and 17 mg; and, Teknar 56 and 80 μl. Bactimos had the highest potency in terms of ITU, followed by Teknar and Deltox, in laboratory tests.

In Rameswaram, the number of anopheline larvae in five samples in the untreated pits ranged from 201 to 510 first and second instars, 32 to 189 third instars, 55 to 120 fourth instars and 3 to 100 pupae on different days of observations, while the number of culicine larvae ranged from

154 to 976, 46 to 204, 27 to 139, and 7 to 154 respectively. In pits treated with 1 g dose of Deltox, there were 213 first and second instars, 126 third instars, 81 fourth instars and 4 pupae of anophelines while there were 210 first and second instars, 207 third instars, 214 fourth instars and 6 pupae of culicines on the pre-spray day. Application of Deltox caused a drastic reduction in the number of different larval instars, on the first day, the reduction being in the range of 98-99 per cent. No pupa was found from the second day after treatment. However, the number of first and second instars increased due to continuous oviposition, whereas the third and fourth instars appeared on the third and fourth day. A second application was, therefore, found necessary on the fifth day to prevent pupation. The larval density was reduced as a result of the second application by 83-100 per cent. In pits treated with 2 g dose, the effect was similar to that observed in pits with 1 g dose and there was no marked difference either in the mortality rate of larvae or in the reappearance of immature population as a result of increased dosage.

The results of the large scale trial in Vedaranyam area showed that all the three formulations were effective in reducing the larval density of both anophelines and culicines in both non-polluted and polluted waters within a period of 24 h after application (Tables I to IV). The *p*H of the water in the pits ranged from 6·5 to 9·0 and the water temperature varied between 36° and 40°C.

In non-polluted pits, the mortality rates of anopheline larvae in the pits treated with Deltox ranged from 54-89, 93-100, 80-100 and 100 per cent for first, second, third and fourth instars respectively. With Bactimos treatment the percentage reduction was 25-95 among first instars and 100 per cent reduction among other larval instars whereas with Teknar treatment there was a reduction of 29-96 per cent in first instars, 80-100 per cent in second instars, 83-100 in third instars and 100 per cent in fourth instar larvae. The rate of reduction for culicine larvae ranged from 0 to 100 per cent in first instars, 75-100 per cent in third instars and 100 per cent in second and fourth instars due to the application of Deltox; 0-100 per cent reduction among first instars and 100 per cent among the other larval instars in pits treated with Bactimos; and 83-100 per cent in first three larval stages in pits treated with Teknar. No IV instar larva was found in pits with Teknar treatments throughout the study period.

In polluted pits the range of per cent mortality of anophelines in the pits treated with different formulations was as follows : Deltox : 37-90, 93-100, 77-100 and 60-100 for I, II, III and IV instars respectively; Bactimos : 0-100 I instar, 50-100 II instar and 100 III and IV instar; and Teknar : 82-100, 92-100 and 100 per cent in different instars. In the case of culicines, the reduction in the pits treated with Deltox was 0-100 per cent in I instars, 75-100 per cent in II and III instars and 67-100 per cent in IV instars, whereas in Bactimos treated pits the per cent mortality ranged from 14-100, 89-100, 50-100 and 67-100 in I, II, III and IV instar larvae respectively. The per cent reduction in larval density in pits treated with Teknar ranged from 75 to 100 for first instars and 100 per cent for other larval instars. Comparison of the mean percentage reduction of different formulations showed that there was no

Table 1. Mean number of anopheline larvae and pupae per pit (total number in five dips) in non-polluted water on different days

No. of pits sampled days	Untreated 40					Treated														
						Deltox (1g/10 m²) 64					Bactimos (1g/10 m²) 8					Teknar (3 m 1/10m²) 18				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
1*	80	41	10	4	1	130	62	19	10	2	156	62	8	2	1	18	10	5	3	1
2	73	36	12	4	1	30	0	0	0	1	5	0	0	0	2	3	0	0	0	1
7*	42	24	15	5	3	42	20	12	2	1	50	8	15	3	1	27	17	6	1	0
8	41	17	12	8	2	5	0	0	0	0	5	0	0	0	0	1	0	1	0	0
12*	27	14	7	3	2	37	16	5	1	0	25	46	2	0	0	11	9	2	0	0
13	26	13	5	3	1	4	1	1	0	0	2	0	0	0	0	2	0	0	0	0
17*	45	12	5	3	1	35	10	2	1	0	12	5	1	1	0	7	5	3	1	0
18	48	12	5	1	1	5	0	0	0	0	9	0	0	0	0	5	1	0	0	0
22*	45	30	11	5	1	36	12	6	1	0	26	14	4	0	0	16	16	7	1	0
23	40	15	11	3	1	10	0	0	0	0	4	0	0	0	0	2	2	0	0	0
27*	48	15	5	3	1	26	14	6	2	0	19	10	2	0	0					
28	36	10	9	2	1	12	1	0	0	0	2	0	0	0	0					
32*	33	34	14	7	1	31	17	5	3	0	11	6	4	1	0					
33	43	49	18	7	2	7	0	0	0	1	3	0	0	0	0					

*Indicates treatment days

Table II. Mean number of culicine larvae and pupae per pit (total number in five dips) in non-polluted water on different days

No. of pits sampled	Untreated 40					Treated														
						Deltox (1g/10 m²) 64					Bactimos (1g/10 m²) 8					Teknar (3ml/10 m²) 18				
days	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
1*	6	3	2	1	1	17	9	4	4	1	10	5	4	3	0	6	18	3	0	0
2	6	5	3	3	0	3	0	1	0	0	3	0	0	0	0	1	0	0	0	0
7*	6	2	2	0	1	2	3	2	1	0	1	1	1	0	0	19	6	1	0	0
8	8	4	8	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
12*	10	2	3	0	1	2	1	1	1	1	2	1	0	0	0	11	5	6	0	0
13	7	2	1	0	0	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0
17*	12	2	1	1	0	3	2	1	1	0	0	0	0	0	0	12	4	2	0	0
18	10	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22*	8	3	2	0	0	2	1	1	1	0	1	1	0	0	0	5	2	2	0	0
23	9	11	3	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
27*	7	2	1	0	0	2	2	1	0		3	1	0	0	0					
28	5	2	1	1	0	1	0	0	0	0	0	0	0	0	0					
32*	6	3	1	1	0	1	1	1	0	0	11	1	0	0	0					
33	19	2	1	1	0	0	0	0	0	0	0	0	0	0	0					

*Indicates treatment days

Table III. Mean number of anopheline larvae and pupae per pit (total number in five dips) in polluted water on different days

No. of pits sampled days	Untreated 10					Treated														
						Deltox (1g/10 m²) 24					Bactimos (1g/10 m²) 5					Teknar (3ml/10 m²) 2				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
1*	25	52	10	3	0	38	21	13	6	1	13	17	7	5	0	9	10	1	4	1
2	41	23	7	5	4	24	1	1	0	1	13	0	0	0	0	0	0	0	0	1
7*	42	24	18	2	0	38	29	15	5	0	11	2	1	0	0	17	1	2	0	0
8	46	22	12	6	3	11	2	3	2	1	13	1	0	0	0	3	0	0	0	0
12*	20	14	3	1	1	31	9	3	1	0	4	4	0	0	0	35	12	1	0	0
13	24	23	15	4	13	3	0	0	0	0	0	0	0	0	0	6	1	0	0	0
17*	42	11	5	3	1	38	16	3	1	0	11	0	0	0	0	52	24	13	2	0
18	20	5	2	2	0	16	1	1	0	1	1	0	0	0	0	8	0	0	0	0
22*	23	7	5	2	0	40	11	4	2	0	10	2	1	0	0	14	7	9	3	0
23	33	4	4	1	1	12	0	0	0	0	63	0	0	0	0	0	0	0	0	0
27*	28	3	3	1	0	37	10	7	4	0	78	31	18	28	0					
28	13	2	1	1	0	10	0	0	1	0	11	0	0	0	1					
32*	22	13	8	1	0	28	8	3	1	0	133	82	106	136	0					
33	12	7	3	2	0	5	0	0	0	0	8	0	0	0	0					

*Indicates treatment days

Table IV. Mean number of culicine larvae and pupae per pit (total number in five) in polluted water on different days

No. of pits sampled days	Untreated 10					Treated														
						Deltox (1g/10 m^2) 24					Bactimos (1g/10 m^2) 5					Teknar (3ml/10 m^2) 2				
	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P	I	II	III	IV	P
1*	23	12	7	10	8	15	51	13	7	1	15	9	2	3	5	3	1	0	2	0
2	20	11	11	6	3	8	1	1	1	1	0	0	0	0	1	0	0	0	0	0
7*	25	9	7	8	1	24	9	4	3	2	14	3	1	3	6	4	2	0	0	0
8	32	8	21	7	1	8	2	1	1	1	0	0	1	0	3	1	0	0	0	0
12*	20	17	3	3	0	13	5	3	1	0	3	9	5	5	2	11	10	5	1	0
13	15	4	15	7	2	1	0	0	0	1	0	0	0	0	5	1	0	0	0	0
17*	31	8	5	3	1	9	4	3	3	1	7	1	2	3	4	6	10	3	1	0
18	30	8	3	5	1	2	0	0	0	1	6	0	1	1	5	0	0	0	0	0
22*	18	10	4	1	1	7	4	4	3	2	56	27	24	39	19	8	0	1	1	0
23	16	8	4	3	1	7	1	0	0	1	17	3	0	0	3	0	0	0	0	0
27*	8	4	0	1	0	13	4	1	2	12	17	6	8	6	3					
28	4	2	0	1	0	0	0	0	0	2	2	0	0	0	4					
32*	5	6	3	3	1	17	11	26	10	1	18	15	7	3	1					
33	5	3	1	7	1	1	1	0	0	1	5	1	0	1	3					

*Indicates treatment days

significant difference in their effect on anopheline or culicine larvae in polluted ($F=0\cdot85$, $P>0\cdot05$; $F=2\cdot08$, $P>0\cdot05$ respectively) and non-polluted water ($F=0\cdot09$, $P>0\cdot05$; $F=0\cdot21$, $P>0\cdot05$ respectively).

In all treatments, relatively a lower larvicidal effect due to *B. thuringiensis* H-14 was observed on first instar larvae, as compared with other larval stages. This may be due to the fact that early first instars do not normally feed for about 2-3h after hatching and there has been daily egg laying in the pits. In the laboratory studies[3] and in some field experiments[2], it has been shown that *B. thuringiensis* H-14 is more effective against I and II instar larvae as compared to III and IV. But this study shows that the formulations are not effective against the early first instars. The larval density gradually increased from the second day after treatment and IV instars appeared by the fifth day. Pupae appeared by the sixth day after the first treatment and this necessitated the application of *B. thuringiensis* H-14 once in 5 days, for effective supression of pupal production. Reappearance of 1st instar larvae by 48 h post-treatment, and the resulting increase in their density thereafter indicated that none of the three formulations tested had persistent larvicidal activity beyond 24 h after application. This was found to be the case in other studies also[2–5].

It has been reported that *B. thuringiensis* H-14 failed to cause 100 per cent mortality among mosquito larvae present in polluted environments and in habitats with heavy vegetation and floating algae[6,7]. However, in the present study, it was observed that *B. thuringiensis* H-14 induced mortality up to 100 per cent in pits with polluted water without any algae and other debris.

While the laboratory studies showed that Bactimos was six times more potent, and Teknar about $1\cdot25$ times more potent than Deltox, in terms of ITU, under field conditions, both Bactimos and Teknar were found to require higher dosages than indicated by the potency (ITU/mg), indicating that there is no relationship between the potency observed in laboratory tests and the field performance of these formulations. This view has been generally accepted by the World Health Organization also. The efficacy of the formulations may differ in relation to the particle size and release rate of toxins. In a recent trial near Delhi[2], it was shown that the dosage/hectare of Teknar was three times the indicated dosage for effective kill of larvae.

The present study clearly showed that *B. thuringiensis* H-14 is effective in the initial suppression of both anopheline and culicine larval populations in coconut garden pits breeding mosquitoes. But no residual activity was observed indicating that *B. thuringiensis* H-14 has to be applied every 5 or 7 days depending on the duration of pupal development. There is thus no obvious advantage in using *B. thuringiensis* H-14 in an operational programme, as compared to other conventional larvicides in use at present, unless improved formulations are available. However, in certain situations where no chemical larvicides can be used, *B. thuringiensis* H-14 could be considered, since this produces an effective kill within 24 h. The formulations available at present have to be improved further so that their

potency and residual action is enhanced and the frequency of application of the formulation is reduced. The study also showed that indigenous formulations are as effective as other commercial formulations. This will also considerably reduce the cost which is an important consideration in operational programmes.

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Recycling potential of *Bacillus sphaericus* in natural mosquito breeding habitats

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Experiments were conducted to examine the recycling potential of two strains (B·6 and B.64) of *B. sphaericus* a mosquito larvicide, in natural mosquito breeding habitats. Both the strains were tested against *Anopheles subpictus* and *Culex sitiens* breeding in polluted coir pits and unpolluted earthworm collection pits respectively. Larvicidal action was observed up to 24 h only. In spite of repeated applications (13 times in coir pits and 9 times in earthworm collection pits), the bacterial agent did not recycle at a level high enough to continuously suppress mosquito larval population.

Microbial control of vectors is being suggested as an adjunct in Integrated Mosquito Control Programmes. In recent years, research on the two larvicidal bacilli *viz.*, *Bacillus thuringiensis* H.14 and *B. sphaericus* had been receiving high priority. A desirable attribute for a biocontrol agent is the "recycling potential" for reducing operational costs. A few reports have indicated that *B. sphaericus* could persist/recycle in the ecosystem and suppress larval populations after one or two introductions[1,2]. The present study was aimed at investigating the recycling potential of two indigenous strains of *B. sphaericus*.

Material & Methods

Two strains (B. 6 and B.64) of *B. sphaericus* serotype H.5 a and b isolated from indigenous sources and developed at the Vector Control Research Centre (VCRC), Pondicherry, were used for the study. Identification of the strains were confirmed earlier by Dr H. de Barjac, Institute Pasteur, Paris.

Coconut husk retting pits (coir pits) and earthworm collection pits present on the banks of Chunnambar estuary near Pondicherry were chosen for the study. The size of coir pits ranged from 2-10 m^2 which had highly polluted saline water and the depth of water column was 30-60 cm. The area of earthworm collection pit ranged from 0·2-0·5 m^2 which had clear water to a depth of 15-30 cm. Both the coir pits and earthworm collection pits supported heavy breeding of *Culex sitiens* and *Anopheles subpictus*.

The WDP formulations of the *B. sphaericus* strains developed at the VCRC were used for the field trials. The formulations of the strains B.6 and B.64 had

LC_{90} values of 200 and 100 μg respectively against *C. quinquefasciatus* third instar larvae[3]. The strain B. 6 was applied weekly for 13 wk to nine coir pits at the rate of 3g formulation, equivalent to 2400 mg of wet biomass per $10m^2$ of water surface at weekly intervals. The formulation was suspended in water at the rate of 3 g/l and sprayed with a knapsack sprayer. Eight coir pits were left unsprayed as control. From 14th to 22nd wk, the coir pits were stirred up every week to make the bottom sediments remain suspended in the water column so that the settled *B. sphaericus* spores/cells, if any, were available for ingestion by mosquito larvae.

The formulation of strain B.64 was applied to earthworm collection pits at the rate of 2 g, equivalent to 1600 mg of wet biomass, per $10m^2$ of water surface. Eight earthworm collection pits were treated with the formulation while 12 earthworm collection pits were left unsprayed as control. The bacterial agent was applied 9 times to the same pits at weekly intervals.

Larval sampling was done in the experimental pits by taking random dipper samples (300 ml water) on four or five occasions during a week and the larvae and pupae present in 5 dipper samples were pooled, counted, recorded instarwise and released back into the respective pits. However, data collected on the day of spray and 24 h later only are presented in the Tables.

Results & Discussion

Effect of B. sphaericus on the mosquito larval populations present in coir pits and earthworm collection pits : In coir pits which normally support breeding of *A. subpictus* and *C. sitiens*, *B. sphaericus* B.6 strain was applied once a week for 13 wk and larval mortality assessed 24 h after application of the formulation. Mortality ranging from 67 to 100 per cent was observed in early (I and II) and late (III and IV) instar larvae of *A. subpictus* (Table I). In the case of *C. sitiens*, in the first eight weeks, reduction in the larval density of early instars ranged from 76 to 100 per cent. Even though the formulation was applied in the following weeks also, the reduction observed ranged only from 0 to 37 per cent. The loss of larvicidal efficacy of *B. sphaericus* in the later part of the experiment may be due to dilution of the pits with sea water during high tides, as these pits are located on the banks of an estuary near the sea coast. A similar pattern was observed in the reduction of late instars also *i.e.*, 56-93 per cent during the first 10 wk and 4 to 38 per cent in subsequent weeks. The application of B.6 formulation was discontinued after 13 wk and from the 14th to the 22nd wk, and monitoring of larval density was continued; the results showed that there was no difference in the density of larvae between the treated and untreated pits once the treatment was discontinued, indicating that no recycling or persistence of *B. sphaericus* B.6 strain occurred in this habitat.

B. sphaericus (B. 64), a strain with higher virulence[3] was applied weekly in 8 earthworm collection pits for 9 wk and larval density monitored. There was only initial reduction in larval density 24 h after each application, ranging from 91 to 100 per cent for early instars and 87 to 100 per cent for late instars of both *A. subpictus* and *C. sitiens* (Table II). From 48 h

Table 1. Effect of *B. sphaericus* B-6 application on the density of *A. subpictus* and *C. sitiens* larvae breeding in coir pits

Day	*A. subpictus*				*C. sitiens*			
	Treated		Untreated		Treated		Untreated	
	EI	LI	EI	LI	EI	LI	EI	LI
1*	45	34	23	12	272	682	156	427
2	8	9	17	12	5	52	141	397
11*	120	102	44	65	218	533	228	495
12	0	0	71	86	7	35	302	276
18*	63	9	97	101	259	132	232	243
19	0	3	89	50	0	55	182	253
25*	53	28	60	50	325	320	169	192
26	13	5	50	43	34	82	159	179
32*	54	24	49	39	332	153	202	259
33	16	2	39	37	79	67	178	215
39*	81	49	86	84	261	211	279	221
40	27	11	30	51	12	93	142	165
46*	16	9	55	40	274	223	258	196
47	5	2	44	41	66	72	279	216
53*	45	26	152	86	211	247	370	356
54	0	0	138	82	47	34	261	307
60*	5	0	63	58	88	66	188	214
61	10	0	73	42	61	59	175	238
67*	0	0	41	32	111	194	64	133
69	22	0	50	20	126	120	98	157
74*	10	2	33	19	226	308	115	216
75	0	0	24	13	223	294	142	153
81*					99	115	146	200
82					82	38	178	160
88*					237	232	192	157
89					149	100	174	149
118					176	139	207	264
123					290	101	247	263
127					288	229	179	171
131					202	200	189	218
142					153	149	245	215
159					136	147	230	174
166					139	152	237	183

*Spray day; EI, early instar; LI, late instar

post application the larval population recovered to the pre-treatment level until another spraying was done.

The coir pits had 89 mg of suspended solids per litre and 2·5 per cent of organic carbon. The water in earthworm collec-

Table II. Effect of *P. sphaericus* B-64 application on the density of *A. subpictus* and *C. sitiens* breeding in earthworm collection pits

Day	*A. subpictus*				*C. sitiens*			
	Treated		Untreated		Treated		Untreated	
	EI	LI	EI	LI	EI	LI	EI	LI
1*	103	61	184	71	101	62	55	17
2	4	4	155	89	1	6	146	61
7*	124	37	66	45	183	58	136	26
8	0	0	38	22	0	0	85	48
14*	105	44	151	54	75	37	61	24
15	0	0	96	85	0	0	54	39
21*	56	41	43	43	35	38	23	75
22	0	0	41	32	0	0	60	86
28*	89	43	36	36	17	45	59	103
29	0	0	36	24	0	0	36	90
35*	56	19	35	48	112	104	66	101
36	0	1	56	66	0	0	68	82
42*	27	9	45	16	38	54	56	111
43	0	0	7	10	0	0	44	79
49*	76	31	30	16	96	59	78	111
50	0	0	27	15	0	0	121	89
56*	193	35	78	30	140	44	136	79
57	18	0	31	22	108	0	178	138

*Spray day; EI, early instar: LI, late instar

tion pits did not have any organic pollution. The salinity was about 30,000 ppm in both coir pits and earthworm collection pits. This study showed that *B. sphaericus* can withstand high pollution and salinity and therefore can be used for larvicidal purposes in such situations.

It has been reported by some that *B. sphaericus* strains may persist/recycle and consequently cause continuous suppression of larval population in treated sites[1,2] (unpublished data from VCRC, Pondicherry). However, in the habitats treated with both strains of *B. sphaericus* (B. 6 and B.64), the pattern of resurgence of larval population indicated that this bacterial agent gives only an initial kill and does not recycle. Our study confirmed that B. 64 is more virulent in inducing higher mortality in the field, as had been observed earlier in the laboratory.

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Indigenous isolation of F-17—an oomycetous fungus pathogenic to mosquito larvae

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A oomycetous fungus was isolated indigenously and was found pathogenic to mosquito larvae. It grew well in Sabouraud broth at *p*H 7·5. It produced a higher number of zoospores when cultured in sunflower-seed-extract incorporated Z medium. The *p*H range of 5·5-6·0 was the best for maximum production of zoospores. The fungus readily infected both culicine and anopheline larvae. Early larval stages were more susceptible to the fungus than late stages. The fungus (F-17) caused total mortality of larvae when released in a polluted cess pit breeding *Culex quinquefasciatus*. It suppressed fourth instar larval and pupal production up to a period of 40 days even though there was daily egg laying.

There is now an increasing awareness that natural enemies could play a significant role in reducing the population density of mosquito vectors of diseases like malaria and filariasis. The Vector Control Research Centre (VCRC), Pondicherry has been carrying on a programme of research on the isolation and development of the promising indigenous biological control agents. Several biocontrol agents have been developed and field tested in the past, but their larvicidal activity was persistent for only a short period. *Lagenidium giganteum*, an aquatic fungus closely related to F-17 has been reported to recycle and infect mosquito larvae in rice fields year after year[1]. Studies were carried out on the cultural requirements of this strain and its larvicidal potential on different vector and non-vector mosquitoes assessed.

Material & Methods

The oomycetous fungus (VCRC acc. no. F-17) was isolated from mermethid nematodes (*Romanomermis iyengari*). The fungus was cultured and maintained on Rose Bengal agar medium.

Biomass production : The influence of different media and *p*H on the biomass production of the fungus was investigated. Rose bengal broth (RBB), Sabouraud dextrose broth (SADB), Sabouraud broth (SAB), brain heart infusion broth (BHIB), potato dextrose broth (PDB), malt extract broth (MEB), KMV broth (modified Jishniac's medium)[2], and peptone yeast extract glucose broth (PYG) were used. Sabouraud broth was pre-adjusted to different *p*H levels, *i.e.*, 5·0, 5·5, 6·0, 6·5, 7·0, 7·5 and 8·0 by using 0·1 N NaOH

or HCl. The media were inoculated with mycelial discs of the fungus and incubated at 28±2°C for 8 days. The flask contents were filtered through Whatman filter paper No. 40 and the biomass thus collected was dried under infrared light at 60°C for one hour and weighed.

Zoosporogenesis : Attempts were made to produce maximum concentration of zoospores per unit volume of media. The method followed was similar to those used by others with slight modification[3]. As there were certain restrictions on the availability of hemp seeds (*ganja*) alternative materials *viz*., seed extracts of sunflower (*Helianthus annus*), brinjal (*Solanum melongena*), tomato (*Lycopersicum esculentum*) and *S. xanthocarpum* and soya oil were added as supplements to the medium. The seeds were dried and powdered. Water extracts of the powders were prepared and added into 'Z' medium in place of hemp seed extract. The sterilized media were poured into petridishes and inoculated with the fungus. The cultures were incubated at 28±2°C for 4-6 days. Then the individual agar pads were removed from the petridishes and transferred to flasks containing 500 ml of sterile distilled water and incubated. Zoospore counts were made at 18, 24, 36 and 48 h of incubation and were used for bioassay.

The effect of *p*H of water on the zoospore production was also investigated. The mycelial agar pads grown on 'Z' medium containing sunflower seed extract were immersed in 500 ml of sterile distilled water whose *p*H was preadjusted to 5·5, 6·0, 7·0, 8·0 and 9·0 as previously stated. The flasks were incubated at room temperature and zoospore count was made at 50th h.

Pathogenesis : Susceptibility of different larval instars of important mosquito species to the fungual infection was determined in laboratory bioassays. Dead larvae from bioassay bowls were daily examined under microscope for production and attachment of zoospores, mode of their entry into host and development of mycelia in the coelomic cavity.

Age of culture, virulence and dose response : To study the impact of age of culture on the virulence of the fungus, the cultures on PYG agar were incubated at 37°C for different periods and bioassayed for larvicidal activity. Experiments were also carried out to find out the influence of different doses of the inoculum on the larval mortality.

Bioassays : Mycelial discs of 0·07 cm^2 from 4-6 days old cultures were used as infective units in all the experiments. Bioassays were conducted in bowls containing 250 ml of chlorine-free tap water with 50 larvae and each treatment had four replicates unless and otherwise specified. For each experiment/treatment appropriate controls were kept. The larvae were fed with sterilized dog biscuit and yeast powder. The dead ones were microscopically confirmed for the fungal infection and larval mortality was scored 3 days after inoculation[4].

Preliminary field evaluation : A cess pit with heavy breeding of *C. quinquefasciatus* was chosen to evaluate the larvicidal efficacy of the agent in natural conditions. The pit measured 60×60×90 cm and had 110 litres of polluted sullage water and was formerly being treated weekly with fenthion to control mosquito emergence. Therefore, at the time of treatment with

fungus, only eggs, I and II instar larvae were present. As the fungus isolate was evaluated in the field for the first time, the dosage had to be estimated by repeated trials. The *p*H, salinity and temperature of the sullage water were recorded and larval density was daily monitored using a standard dipper.

Results & Discussion

Biomass production : The results on the fungal growth are presented in Table I. BHIB and SAB (without dextrose) supported maximum biomass production, *i.e.*, 145·8 and 143·8 mg respectively, per 100 ml of broth. Presence of malt extract in the medium totally inhibited the growth. Though BHIB and SAB yielded almost same quantity of biomass, it is more economical to use SAB. A maximum biomass production of 179·8 mg/dl was obtained from SAB adjusted to a *p*H of 7·5. The biomass production was reduced at higher or lower *p*H, indicating that the optimal range was at near neutral *p*H.

Table I. Effect of different media and *p*H on the biomass production of the fungus (F-17)

Culture media	Biomass mg/dl*	*p*H of SAB broth	Biomass mg/dl**
RBB	34·2	5·0	37·7
SADB	39·7	5·5	76·1
BHIB	145·8	6·0	81·1
SAB	143·8	6·5	142·7
PDB	17·4	7·0	143·8
MEB	00·0	7.5	179·8
KMVB	24·2	8·0	141·9
PYG	30·2		

*Mean of 6 replicates; **Mean of 3 replicates; RBB, Rose bengal broth; SADB, Sabouraud dextrose broth; BHIB, Brain heart infusion broth; SAB, Sabouraud broth; PDB, Potato dextrose broth; MEB, Malt extract broth; KMVB, KMV broth; PYG, Peptone yeast extract glucose broth

Zoosporogenesis and virulence : Use of hemp seed extract in 'Z' medium has been found to induce production of higher concentrations of zoospores[5]. In the present study the incorporation of sunflower, brinjal, tomato and *S. xanthocarpum* seed extracts and soya oil stimulated production of zoospores to a maximum of 14×10^4, $4\cdot2\times10^4$, $1\cdot1\times10^4$, $0\cdot3\times10^4$ and $6\cdot1\times10^4$ per ml respectively. It has been reported that *Lagenidium* produced $1\cdot7$-$3\cdot8\times10^7$ zoospores per 10 cm^2 area of mycelial disc[1]. Maximum production of zoospores varied with different media. Sunflower and brinjal seed extracts induced maximum number of zoospore production when incubated for 36 h, where as soya oil and tomato seed extract induced spore production only when incubated for 48 h. Zoospores obtained from sunflower seed extract and soya oil incorporated 'Z' medium caused a maximum of 73-74 per cent larval mortality where as the ones obtained from tomato and brinjal seed extract incorporated 'Z' medium caused only 43 and 24 per cent larval mortality respectively. The study showed that the incorporation of sunflower seed extract should be preferred to others for maximum production of zoospores.

The fungus produced zoospores in distilled water, at all the *p*H levels (5·5, 6·0, 7·0, 8·0 and 9·0) tested. At the *p*H range of 5·5-6·0, 1×10^4 to $1\cdot25\times10^4$ zoospores were produced per ml compared with $0\cdot37\times10^4$–$0\cdot50\times10^4$ zoospores produced per ml at the *p*H range of 7·0-9·0. The results clearly show that the

fungus produced more number of zoospores at 5·5-6·0 *p*H range.

Pathogenesis : Biflagellate, pear shaped zoospores were produced and released from mycelial discs, which encysted on the cuticle of mosquito larvae. The mouth and the external aperture of respiratory siphon also acted as portals of entry of the fungus (Fig. 1). Blockade of respiratory siphon by mass of zoospores was observed. Some of the zoospores were observed attaching to the inner wall of the alimentary tract. Hyphal strands proliferated and filled the haemocoel of larvae within 72 h of exposure. Sporangia were formed by segmentation of hyphae. Discharge tubes arose from sporangia and protruded outside dead larvae through intersegmental regions. At the end of the discharge tubes, vescicles containing zoospores were formed. After active movements for some time, the zoospores were released in the surrounding medium. The progress of infection and the symptoms observed in this study were similar to that reported by others[6,7].

Susceptibility of different species : All stages of *Culex quinquefasciatus*, *C. tritaeniorhynchus*, *C. sitiens*, *Anopheles stephensi* and *Aedes aegypti* larvae were infected by the fungus (Table II). *C. sitiens* was most susceptible (95% mortality) and *Ae. aegypti* was the least susceptible one (48% mortality). Generally culicines appeared to be more susceptible to the fungus than anophelines. Early larval instars of all the mosquito species were more susceptible than fourth instars. In the case of *C. quinquefasciatus*, 67 per cent of the I instar larvae died due to fungal infection while only 11 per cent died among fourth instars. Similar trend was observed among the first and fourth instars of *C. tritaeniorhynchus* and *Ae. aegypti*. Similar results were obtained in another study when 90 per cent mortality occurred among 4 day-old *C. restuans* larvae and only 5 per cent mortality occurred among 10 day-old larvae[6]. The fungus grows rapidly in the larva. Others have found that *Lagenidium* infected larvae loose their metabolites rapidly[8] and concluded that the primary cause of larval death might be due to deprivation of infected larvae of metabolites by the fungus. Most probably the physiological stress exertred by the fungus upon late larval stages is less severe than that on early larval stages. This may be one of the reasons for finding less mortality in IV instars when exposed to infection.

Age of the culture versus virulence : Total mortality of larvae occurred when they were exposed to four day old-fungal cultures. The mortality rate came down to 75 when exposed to 8 day old cultures and it was further reduced to 4 when exposed to 15 day old cultures. It has been reported by others that zoospore production declined to 28·5 per cent by 11th day when compared with that produced on fourth day[9]. The ability of mycelia/hyphae to produce zoospores may be linked with nutritional factors.

Dose response : In the present study 78 to 86 per cent larval mortality was obtained with doses ranging from 1 to 6 discs and there was no correlation between the dose used and larval mortality observed. These results are similar to that observed by Goettol *et al*[10] and are contradictory to the observations by Umphlett and Huang[6]. It was observed that when the fungus infected/dead larvae were not

Fig. 1. Oomycetous fungus (F-17). (*a*) Zoospores found attached to the anal papilla of *C. quinquefasciatus* larva prior to initiation of infection (*b*) The fungus infected *C. quinquefasciatus* larva showing ramification by hyphae; (*c*) Zoospores formed on an infected larva and ready for release.

Table II. Mortality of different larval instars when exposed for 3 days to the fungal infection in laboratory

Larval instars	Percentage mortality
C. quinquefasciatus	
I	79
II	72
III	27
IV	11
C. tritaeniorhynchus	
I	55
II	78
III	38
IV	34
C. sitiens	
I	95
II	79
III	71
IV	65
Ae. aegypti	
I	48
II	4
III	7
IV	3
A. stephensi	
I	50
II	72
III	60
IV	58

removed from the bioassay bowls, zoospores were produced and released by the infected larvae and cadavars. The resulting infectious zoospores continued to infect other larvae over a period of time as indicated by the absence of a normal dose response curve.

Preliminary field evaluation : The results of the field test are illustrated in Fig. 2. The experimental pit chosen for the study had considerable organic pollutants. The water salinity was 200 ppm and *p*H was 6·8-8·0. The water temperature varied between 23° and 28°C. *C. quinquefasciatus* was breeding in the pit and 33 first and second larval instars were present per dip. Microscopic examination and culturing of pretreatment larval samples did not yield any mosquito pathogenic microbial agent. All the larvae present in the pit were killed within 48 h of the first application of fungus. Dead larvae were examined for infection and the fungus (F-17) was reisolated from them. Due to daily oviposition first instar larvae were seen on the third day. Throughout the study period the density of first, second and third instar larvae fluctuated but there were no fourth instar larvae or pupae seen throughout the study period of 42 days. Other studies show that *L. giganteum* persisted/recycled and effectively checked mosquito larval populations in rice fields over several years[11,12]. The results show that the larvae hatching from daily egg laying get infected, and the infection results in the death of these before reaching IV instar and subsequently pupation. Also it was observed that the fungus got established in the treated site and was producing and releasing fresh batches of zoospores by utilizing the mosquito larval cadavers and the organic pollutants as sources of nourishment.

The fungus has been found capable of infecting larvae of a wide range of vector mosquitoes tested. It grew very well on simple media. The fungus infected larval cadavers supported the production of additional infective units *i.e.*, zoospores.

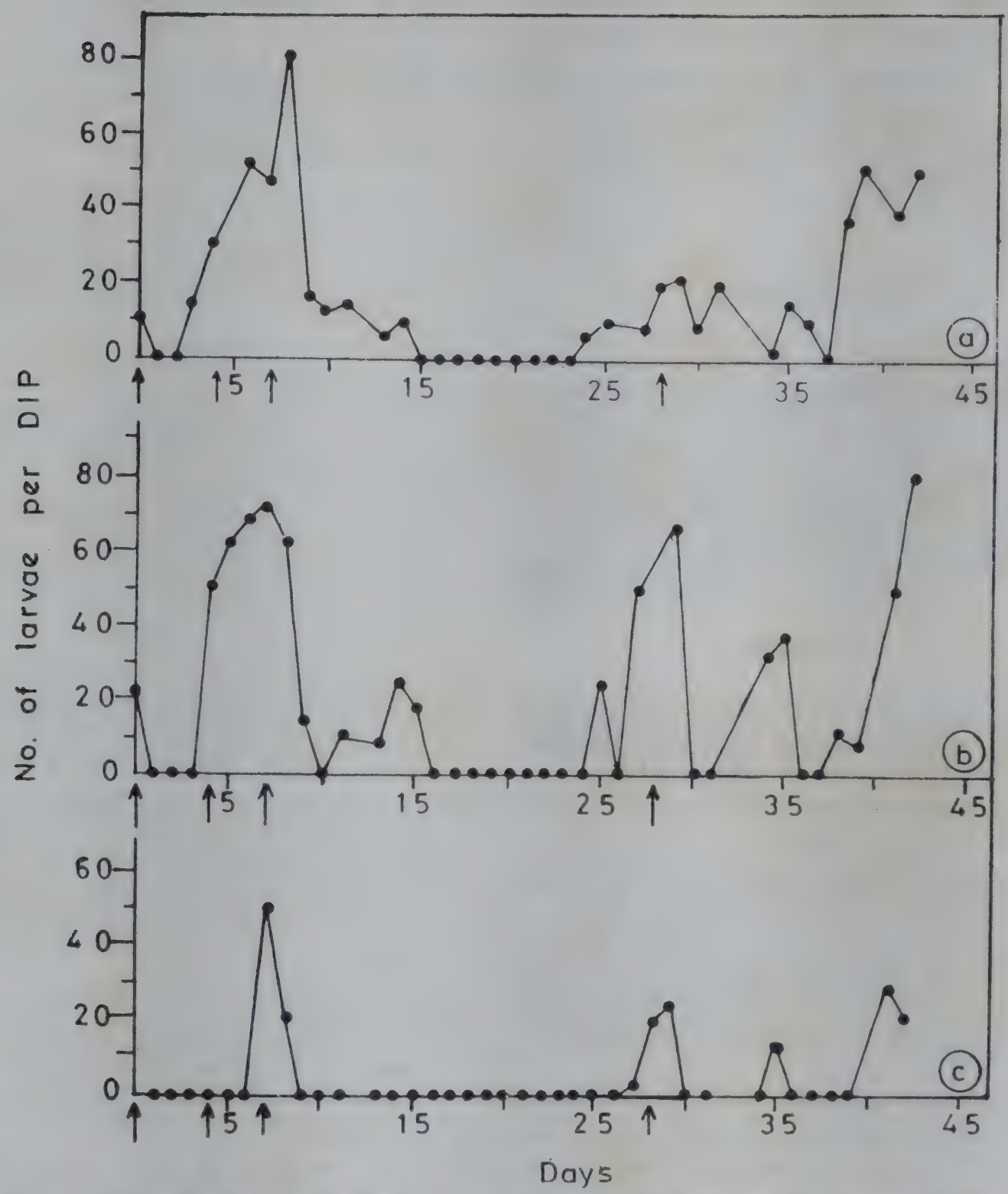

Fig. 2. Effect of the fungus on *C. quinquefasciatus* larvae (*a*) I instar (*b*) II instar (*c*) III instar. Days on X-axis indicate days of application of fungus. No IV instar or pupae developed.

The fungus was able to recycle and continuously suppress pupal production in a polluted mosquito breeding habitat for a period up to 40 days. None of the available isolates of *L. giganteum*, which is closely related to the fungus (F-17) reported[13] are known to tolerate pollution. The fungus does not retain virulence when stored as mycelia or zoospores for more than a week. Further investigations are required on the techniques of mass production and storage to enable large scale

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field trials to suppress vector population. This indigenous fungal isolate appears to have the potential for development and use in an operational programme to continuously suppress vector breeding in polluted habitats after an inoculative release.

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Antibiotic sensitivity of *Plasmodium falciparum in vitro*

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Efficacy of 11 antibiotics was tested *in vitro* against two strains of *P. falciparum* sensitive to chloroquine and pyrimethamine and one strain resistant to both the antimalarial compounds. Ampicillin, streptomycin, kanamycin, chloramphenicol, erythromycin and chlortetracycline were ineffective, in the concentrations tested. Oxytetracycline, demeclocycline, doxycycline, clindamycin and lincomycin were effective in concentrations equivalent to or less than the human therapeutic plasma levels. Synchronized culture studies suggested that these antibiotics act on the schizonts and that the sensitivity of field isolates can be determined by a 48 h test.

Rapid spread of chloroquine-resistant *Plasmodium falciparum* has led to a search for alternate chemotherapeutic agents. Puri and Dutta[1] have recently reviewed the earlier studies on antibiotics for antimalarial activity *in vivo*. Methods for *in vitro* cultivation of *P. falciparum*[2] and its synchronization[3] now permit to test the stage-specific action of drugs on the parasite directly. We have evaluated, *in vitro*, the efficacy of some antibiotics against two strains of *P. falciparum* sensitive to chloroquine and pyrimethamine and one resistant to them. The results have been compared with a similar study recently reported by Geary and Jensen[4].

Material & Methods

P. falciparum strains, FAN-5, FDN-1 and FRN-1, were adapted to and maintained in continuous cultures as described earlier[5]. When tested in *vitro*, FAN-5 and FDN 1 were sensitive to chloroquine, pyrimethamine, quinine and mefloquine and FRN-1 was resistant to chloroquine and pyrimethamine but sensitive to the other two drugs. Initially, all the antibiotics were screened against the three strains using concentrations equivalent to the maximum serum levels attained after administration of therapeutic doses against bacterial infections[6]. Those antibiotics which were found effective were retested using the maximum, minimum and one-tenth of the minimum serum levels. Dosages were calculated with reference to the volume of 50 per cent erythrocyte suspension in culture.

Efficacy of the drugs on asynchronous cultures was determined by a 96 h test[7] and on synchronized cultures by a 48 h test[8].

Results & Discussion

Eleven antibiotics *viz*., ampicillin, streptomycin, kanamycin, chloramphenicol, erythromycin, chlortetracycline, oxy-

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Table I. Antibiotics against *P. falciparum in vitro* : Results of representative experiments

Antibiotic	Concentration tested (μg/ml of red cell suspension in culture)	Asexual parasite count* per 10,000 RBC									ED_{50} (μg/ml)†		
		FAN-5			FDN-1			FRN-1					
		0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h	FAN-5	FDN-1	FRN-1
Lincomycin hydrochloride	0	56	220	280	44	165	505	40	258	761	4·0	0·7	0·7
	35		160	38		48	38		156	35			
	10		128	70		68	50		190	48			
	1		120	190		95	135		162	218			
Clindamycin hydrochloride	0	56	220	280	44	165	505	60	355	866	0·12	0·13	0·11
	8		93	30		135	125		198	88			
	2·5		145	50		80	70		285	78			
	0·25		103	48		135	110		190	98			
Demeclocyline hydrochloride	0	35	80	114	52	335	810	40	258	761	3·75	NT	0·13
	25		14	16		156	18		180	22			
	2·5		38	64		NT	NT		113	63			
	0·25		68	133		NT	NT		178	395			
Doxycycline hydrochloride	0	55	367	935	44	163	495	40	258	761	0·36	0·86	0·15
	15		278	22		120	153		110	32			
	1·5		265	135		149	225		139	83			
	0·15		253	634		155	293		195	378			
Oxytetracycline hydrochloride	0	65	303	726	52	333	810	56	213	545			
	10		245	73		425	195		148	29			

*Mean of two wells for each reading; †Based on the 96 h readings; NT, Not tested

tetracycline, demeclocycline, doxycycline, clindamycin and lincomycin were tested against the three strains in 99 experiments. The first six drugs were not effective. Lack of inhibitory activity by penicillin and aminoglycosides (streptomycin and kanamycin) is in conformity with the findings of Geary and Jensen[4]. However, our observations with chloramphenicol and erythromycin were different from theirs. This may be due to different methods used for determining the efficacy of the antibiotics. Geary and Jensen[4] determined ED_{50} values which were 7·34 and 1·03 μg/ml of medium respectively. We examined, on the other hand, the inhibitory effect of drugs equivalent to maximum serum levels; 1·3 and 1·0 μg/ml of medium respectively based on our method of calculation. Evidently, at these concentrations, the drugs were not effective in their system also.

Table I depicts representative experiments with the 5 antibiotics effective against asynchronous cultures of the three strains. The ED_{50} values for clindamycin and tetracyclines were much lower than those observed by Geary and Jensen[4] indicating that the method employed in our study was sensitive enough for assaying the slow-acting antibiotics. In most of the experiments involving the effective concentration of drugs, the 48 h counts were greater than inocula although less than those of controls. However, inhibition was apparent when further incubated in a drug-free medium, as evidenced by the 96 h readings. These results indicate that 48 h treatment was adequate, though the action was demonstrable on further incubation in drug free medium. That this could be due to asynchronous nature of cultures was supported by the experiments using synchronized cultures (Table II). After sorbitol treatment, the inoculum contained rings and trophozoites. At the end of 48 h incubation, there was an increase in the rings and schizonts resulting in about a 2-fold increase in the total counts. In drug treated wells, there was an increase in the schizonts but decrease

Table II. Effect of antibiotics on synchronized *P. falciparum* (FAN-5) *in vitro*

Antibiotic	Concentration μg/ml of 50% red cell suspension in culture	Asexual parasite count* per 10000 RBC			Total
		Differential			
		R	T	SH	
Inoculum	—	244	16	0	260
Control	—	283	14	141	483
Lincomycin hydrochloride	35	18	20	211	249
Clindamycin hydrochloride	8	66	17	166	249
Oxytetracycline hydrochloride	10	87	17	170	274
Demeclocycline hydrochloride	25	77	20	152	249

*Mean of 4 wells in two experiments; R, Rings; T, Trophozoite; SH, Schizont

in the rings. Total counts also remained almost the same indicating the inhibitory action of drugs. These results suggest that the drugs act on the schizonts. A 48 h reading was adequate, when the inoculum contained early asexual stages. A 48 h test may thus prove useful to assay antibiotic sensitivity of *P. falciparum* 'wild' isolates, as these contain rings and trophozoites of asexual stages in the peripheral blood.

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Loss of resistance of *Plasmodium falciparum* to chloroquine after culture adaptation

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Chloroquine susceptibility of 4 *P. falciparum* isolates was compared before and after adaptation to continuous cultures by a modified Trager and Jensen's candle jar technique. When tested before adaptation by the Rieckmann micro *in vitro* test, the isolates showed resistance. After adaptation to grow in continuous cultures, they were found to be sensitive to the drug, when re-examined by the same test. This was confirmed by a modified Richards and Maples' 96 h test. In one of the strains (FRN-1), which was monitored for chloroquine susceptibility at different passage levels, the loss of resistance was observed to be gradual. These results are in contrast with the reported biological advantage possessed by the chloroquine resistant parasites over the sensitive ones.

After the first report of successful continuous cultivation of blood stages of *Plasmodium falciparum in vitro*[1], a large number of culture lines have been established in several laboratories. Studies have shown that field isolates represent several clones[2], some of which may be selectively favoured under culture conditions. *In vitro* studies also indicate heterogeneity in drug response of field isolate[3]. Therefore, the importance of assessing drug susceptibility of *P. falciparum* isolates before and after adaptation to continuous cultivation has been stressed recently[3]. We therefore undertook a study on this aspect.

Material & Methods

Strains : Blood specimens were collected in heparin (10 IU/ml) from four *P. falciparum* infected patients and designated as FDN-1, FHN-2, FHN-5 and FRN-1. FDN-1 was a Delhi strain from a patient who gave a previous history of non-response to chloroquine treatment. The detailed history of the patient and method of adaptation of this strain to the culture have been reported elsewhere[4]. FHN-2 and FHN-5 were from patients from Haryana. The *in vivo* tests could not be completed as the patients were not avilable for further studies. FRN-1 was an Assam strain from a patient with a history of chloroquine resistance *in vivo*.

The strains were adapted to continuous culture following the candle jar technique of Trager and Jensen[1], except that glass vials were used[5] in place of petridishes. A strain was considered adapted *in vitro*, when parasitaemia increased 5- to 10-fold in 96 h after a sub-culture[6].

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Tests : Before initiating the culture, the four isolates were tested by Rieckmann's micro-test[7] recently standardized[8], using polysterene plates (LAXBRO) charged with graded concentrations of chloroquine. These plates were charged through the courtesy of WHO and compared with the plates supplied in the WHO kit during a WHO sponsored workshop held at NICD, Delhi in March 1982, with reference to two of the above strains (FHN-2 and FHN-5), and the results were found to be in agreement. After adaptation, three of them (FRN-1, FHN-2 and FHN-5) were again tested by micro *in vitro* technique following sorbitol treatment[9] and all four by the 96 h test of Richards and Maples[10] modified in this laboratory[11].

For each type of test with culture-adapted strains, at least 2 experiments were conducted. A culture-adapted chloroquine sensitive strain, FAN 5, which has been examined repeatedly for over 3 yr was included for monitoring the test for chloroquine sensitivity. In micro *in vitro* tests, the growth at 1·14 pmol of chloroquine was considered as indicative of resistance[8], whereas in the 96 h test, 1 pmol was considered as the discriminatory concentration. Drug concentration is expressed in terms of per μl of blood for field isolates or per μl of 50 per cent mixed erythrocyte suspension (MES) in culture[11].

Results

Results of micro *in vitro* tests (Table I) showed that inhibitory concentrations for FDN-1 and FHN-2 were 6·4 pmol and for FHN-5 it was 3·2 pmol indicating resistance to chloroquine. After adaptation, the strains were sensitive, being inhibited by 1·14 pmol. This observation was confirmed by the 96 h test, where in inhibitory concentration was 0·5 pmol for all the three strains (Table II). Thus,

Table I. Sensitivity of *P. falciparum* to chloroquine before and after adaptation to culture by micro *in vitro* test

Strain	Before (B)* After (A)**	Parasite inoculum per μl of blood	Control per 100 asexual parasites	Schizont maturation % of control						
				Chloroquine : pmol/μl of blood or 50% of MES						
				0·2	0·4	0·8	1·14	1·6	3·2	6·4
FDN-1	B	40000	62	NT	44	NT	36	31	NT	0
FHN-2	B	20000	18	88	88	100	94	33	16	0
	A (26)	87000	37	105	113	24	0	0	0	0
FHN-5	B	3905	57	64	50	35	30	26	0	0
	A (18)	93000	56	92	85	7	0	0	0	0
FRN-1	B	50000	29	96	82	65	68	51	10	0
	A (101)	24000	41	87	12	0	0	0	0	0

*'Wild' isolates from patients; **From culture; Figures in parentheses give the passage level; NT, not tested

after adaptation, a shift in the drug sensitivity was apparent.

On the basis of our experience with three strains, the fourth FRN-1 was examined closely. As evident from Table I, when tested before initiating the culture, it was inhibited by 6·4 pmol. Subsequently, in order to monitor the effect of continuous passages on chloroquine susceptibility, 96 h test was employed at selected sub-passage levels. The profile of response depicted in the Fig. shows that the strain was resistant at the outset, lost half of its resistance by about the 8th passage, was completely sensitive around 25th passage level and reached a plateau after

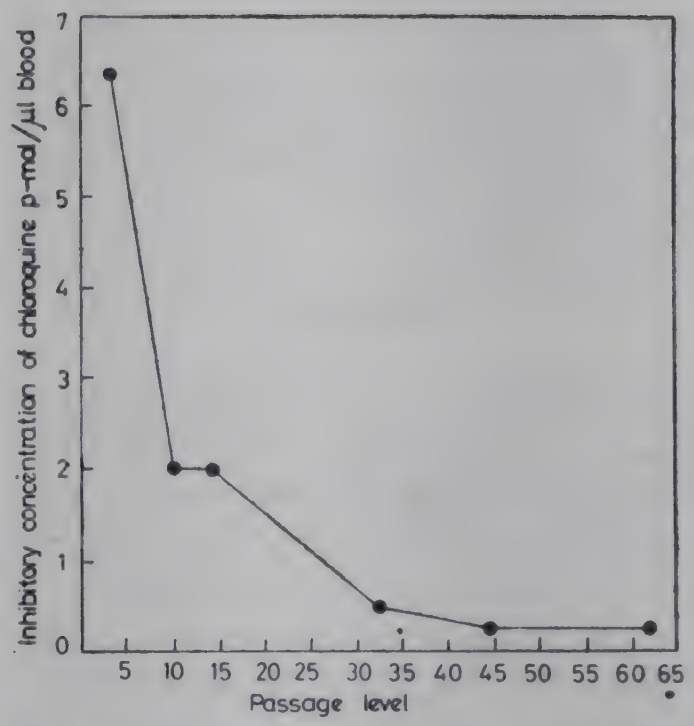

Fig. Response of *P. falciparum* (FRN-1) at different passage levels to chloroquine by the 96 h test.

Table II. Sensitivity of *P. falciparum* to chloroquine after adaptation to culture by the 96 h test

Strain	No. of parasites per 10^4RBC						
	Inoculum (O-h)	Count at (h)	Control	Chloroquine : pmol/µl of 50% MES			
				0·13	0·25	0·5	1·0
FDN-1 (31)	60	48	235	118	NT	7	2
		96	315	123	NT	0	0
FAN-5 (174)	70	48	235	167	NT	5	5
		96	295	164	NT	0	5
FHN-2 (20)	100	48	252	212	102	55	NT
		96	430	480	150	0	NT
FAN-5 (252)	88	48	242	235	117	80	NT
		96	415	520	138	0	NT
FHN-5 (10)	60	48	245	236	224	55	15
		96	505	375	238	12	0
FAN-5 (278)	40	48	207	162	48	15	5
		96	483	245	57	7	0
FRN-1 (62)	30	48	268	222	94	64	24
		96	582	451	27	15	0
FAN-5 (316)	76	48	394	384	240	12	NT
		96	736	708	532	16	NT

Figures in parentheses give the passage levels; NT, Not tested

about 40 passages. These results indicate that the transition was gradual. Further, micro *in vitro* test results at 101st passage level (Table I) also confirmed the sensitivity of the strain.

Discussion

In vitro tests on field isolates (Table I) indicate that although the strains were resistant, there was a proportion of drug sensitive parasite population. From the data on tests after culture-adaptation, it could be inferred that the culture system employed by us appeared to have favoured the growth of sensitive population in preference to the resistant ones. On the other hand, Chin and Collins[6] have recently reported the selection of a resistant variant from a clinically sensitive Haitian-I strain, after adaptation in the absence of chloroquine pressure. The variant was probably selected out of a heterogenous population of parasites. Further Jensen *et al*[12] reported that a line of FCR-3, which was originally chloroquine-sensitive became resistant, after four years of continuous propagation. According to Trager[13], this strain probably contained an appreciable proportion of parasites with reduced sensitivity to chloroquine. It has been pointed out that chloroquine resistant population possesses a biological advantage over the sensitive one[6,14]. However, our finding is in contrast with these observations and cannot be readily explained. The effect of further continuous cultivation on chloroquine sensitivity of the 4 culture-adapted strains reported by us, needs to be further investigated.

It is reported[15] that chloroquine resistance is stable in experimental models and therefore it is possibly so in *P. falciparum* in nature. In the absence of a direct evidence and in the light of our findings, it could be of interest to investigate the effect of withdrawl of chloroquine on the stability of chloroquine resistance in *P. falciparum* in a given area. A protocol for such a study has been recently proposed[15].

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A field test to assay pyrimethamine sensitivity of *Plasmodium falciparum in vitro*

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An *in vitro* micro-test is described for assessing the sensitivity of *P. falciparum* culture adapted strains and field isolates to pyrimethamine. Tests were conducted in microtitre polystyrene plates with 10 μl of blood from *P. falciparum* infected patients or 50 per cent, infected erythrocyte suspension of sorbitol treated cultures, and 200 μl of RPMI-1640 growth medium with or without drug. The plates after sealing with a cellotape were incubated at 37°C for 48 h. Inhibition of parasite multiplication was the basis of the drug assay. Two culture-adapted strains *i.e.*, one sensitive and the other resistant to pyrimethamine and 10 field isolates were tested. Adequate multiplication could be seen in the control wells, for comparison with the growth in the drug treated wells. Difference in the response between the sensitive and the resistant culture-adapted strains was evident. All the field isolates were found to be sensitive. The test system does not need any gassing. Being simple and reproducible, the test offers a new tool for testing the sensitivity of *P. falciparum* to antimalarials under field conditions.

Chloroquine resistance has imposed considerable constraint on the containment of falciparum malaria. In many countries, resistance to pyrimethamine has also been recorded and in some areas there is evidence that a combination of pyrimethamine with sulfadoxine (Fansidar) has become less effective in the treatment of multiple drug resistant falciparum malaria[1]. Development of field tests for monitoring responses of *Plasmodium falciparum* to the currently used as well as potential antimalarial agents has assumed importance, for being forewarned of any change in the susceptibility status in a given area. Rieckmann's *in vitro* micro test[2] for assessing the chloroquine sensitivity of *P. falciparum* has now been standardized for field use[3]. However, a suitable test for determining pyrimethamine sensitivity of *P. falciparum* field isolates is yet to be developed. We have reported earlier[4] a test using the sealing technique for determining sensitivity of field strains to chloroquine. This test was further modified to test pyrimethamine sensitivity to culture-adapted as well as field isolates of *P. falciparum* and the results are presented here.

Material & Methods

Parasites: Cultures of FAN-5 sensitive to chloroquine and pyrimethamine[5] and FRN-1, clinically resistant to these drugs (unpublished data) were maintained by

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serial sub-passages in human A erythrocytes by a modified candle jar technique[6]. RPMI-1640 containing 25 mmol Hepes buffer, 300 mg glutamine and 40 mg gentamycin per litre of medium and *p*H adjusted to 7·2 with 5 per cent sodium bicarbonate solution (complete medium), was used as the culture medium. Field isolates were obtained from patients with patent *P. falciparum* parasitaemia.

Pyrimethamine : A 10^{-2} *M* stock solution of pyrimethamine (Walter and Bushnell) was prepared in 0·5 per cent lactic acid[7] and stored at 4°C.

Testing methods :

(*i*) For culture-adapted strains—Four-days-old cultures with about 5 per cent parasitaemia were treated with 5 per cent sorbitol to select the ring stages[8], washed once with complete medium and resuspended in the same to get 50 per cent suspension. After enumerating the parasite count it was diluted with washed 50 per cent normal human A^+ erythrocytes[6] to get about 1 per cent parasitaemia.

From the stock solution, fresh dilutions of pyrimethamine were prepared in complete medium and tests were conducted in microtitre plates (LAXBRO). After initial experiments to determine the optimum cell-medium ratio, the following procedure was adapted : Each well received 10 µl of parasite inoculum, and 200 µl of complete medium with graded concentrations of pyrimethamine. In the controls, instead of the drug, medium containing lactic acid equivalent to that present in the highest drug concentration well, was used. Two wells for controls and two for each of the drug concentrations were used. They were than sealed firmly with a cellotape (Tixo/Panfix) and incubated at 37°C for 48 h continuously without change of medium or cellotape. After incubation, a thin smear from each well was prepared, stained with Giemsa's stain and number of parasites per 10^4 RBC was enumerated.

(*ii*) Field isolates—Tests were put up in pre-dosed microtitre plates which were prepared as follows : Appropriate dilutions were made in distilled water from pyrimethamine stock solution and delivered to the wells to get 0·1, 0·2, 1,2, 10 and 20 pmol of drug in wells C through H respectively. Control wells A and B were dosed with lactic acid dilution equivalent to that present in the well containing the highest concentration of the drug. The charged plates were dried at 37°C and stored at 4°C till use. When cultures were set up, the drug concentrations in wells C to H were respectively 5×10^{-10}, 10^{-9}, 5×10^{-9}, 10^{-8}, 5×10^{-8} and 10^{-7}*M*.

From each patient, 100 µl of fingerprick blood was collected in heparin (10 IU/ml of blood) after examining the urine of the patient by the Dill and Glazko[9], and lignin[10] tests. Microcultures were set up with 10 µl of blood and 200 µl of complete medium. After brief agitation, the plates were sealed firmly with a cellotape followed by incubation and enumeration of parasites as for tests with culture-adapted parasites.

End point determination : For each specimen, O h parasite count was compared with the 48 h count of control as well as drug containing wells. The minimum concentration of the drug which inhibited parasite multiplication was taken as inhibitory concentration. A strain was consi-

dered sensitive if it was inhibited by 10^{-8} *M* of the drug[11].

Results

In the first series of experiments, the effect of 10-fold increasing concentrations of the drug were tested against FAN-5 (5 experiments) and FRN-1 (2 experiments). Results in Table I showed that in case of FAN-5, the 48 h count in controls was 2·3 to 5·1 fold greater than that of 0 h counts and in case of FRN-1, the increase was 2·4 and 2·5-fold. In all the experiments, FAN-5 was inhibited by 10^{-8} *M* concentration whereas FRN-1 was inhibited by 10^{-5} *M* concentration of the drug. Further, two experiments with each strain employing 1 : 2 to 1 : 16 dilutions from the above inhibitory concentrations indicated that they were inhibited by $2{\cdot}5\times10^{-9}$ *M* and $1{\cdot}25\times10^{-5}$ *M* respectively. The difference in response between sensitive FAN-5 and resistant FRN-1 was evident from these results confirming earlier observations.

Results of tests with 10 field isolates are given in Table II. Parasite count at 0 h ranged from 20 to 140 per 10^4 RBC. After incubation, in the control wells, there was 2·1 to 6·3-fold increase in parasitaemia. Responses to pyrimethamine showed that inhibitory concentrations were

Table I. Effect of pyrimethamine *in vitro* against culture adapted strains of *P. falciparum*

Experiment	Strain and passage level	Asexual parasites per 10^4 RBC at 48 h								
				Pyrimethamine concentration (*M*)						
		Oh*	Control	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
I	FAN-5 P-244	90	207	202	120	85	82	—	—	—
II	FAN-5 P-249	55	210	—	—	50	40	40	—	—
III	FAN-5 P-256	105	378	—	245	82	83	—	—	—
IV	FAN-5 P-265	136	705	816	630	116	104	—	—	—
	FRN-1 P-11	110	280	200	210	200	210	170	80	—
V	FAN-5 P-269	132	453	405	320	85	60	—	—	—
	FRN-1 P-14	70	170	—	—	160	155	145	95	50

*90 to 95% ring stages; —, not tested; Data give the mean values of two wells

Table II. Response of pyrimethamine of *P. falciparum* field isolates by the micro *in vitro* test

Patient*	Asexual parasites per 10^4 RBC at							
			48 h					
	O h		Pyrimethamine concentration (*M*)					
		Control*	5×10^{-10}	10^{-9}	5×10^{-9}	10^{-8}	5×10^{-8}	10^{-7}
Mu	35	120	110	40	45	16	20	20
Ka	140	313	280	220	110	25	50	30
Su	36	84	35	55	30	45	35	18
Ba	102	222	—	160	72	25	40	20
Nm	85	232	120	135	78	76	72	68
Hu	105	505	246	70	90	80	80	66
Mo	20	82	56	15	12	16	16	12
Hk	35	120	96	24	18	25	18	15
Ma†	72	330	—	—	72	—	—	—
Kl	44	280	235	225	15	10	—	—

*Data under control give the mean values of two wells. †Since the quantity of blood from this patient was inadequate, only controls and one drug concentration were used; —, not tested

$5\times10^{-10}M$ for one, 10^{-9} *M* for four and 5×10^{-9} *M* for the rest indicating that all the isolates were sensitive to the drug.

Discussion

The tests described by earlier workers for determining pyrimethamine sensitivity of culture-adapted[12] as well as field isolates[11,13] were based on the candle jar technique for cultivation of *P. falciparum*[14]. This test involves daily gas regeneration during incubation. The test reported by us was developed, following a technique for culture of falciparum parasites in sealed bottles[15]. The present study shows that with the sealed technique, adequate multiplication of culture-adapted as well as field strains could be achieved in controls during 48 h, which enabled a meaningful comparison with growth in drug containing wells. This was achieved without the need for gassing or a change of the medium during incubation. The technique thus offers a simple system to retain the gas produced during parasite multiplication which presumably can meet the required gas tension and help to prevent abnormal shift, in *p*H. Being simple and reproducible, the technique may prove to be a useful tool for testing drug susceptibility of *P. falciparum* under field conditions.

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Feasibility of community participation for vector control in villages

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A three year study was carried out on the feasibility of community participation in a vector control programme in some selected villages of Pondicherry. The study showed that environmental control of vectors is possible without the advent of any high-flown technology for the control of vectors in rural areas. While appropriate technology for vector control can be developed in villages without using chemical insecticides, for enlisting community participation on a sustained basis, it has been found that vector control programmes should be integrated with the overall socio-economic development and health care delivery in the area concerned.

Malaria is endemic in many villages along the Coromandel coast of south India[1,2]. There has been sporadic outbreaks of malaria in some of these villages and in spite of the best efforts of the State Malaria Control organizations it has not been possible to eliminate the foci of infection. A three year study was carried out in four coastal villages of Pondicherry to explore the feasibility of environmental control of vectors for controlling vectors of malaria with community participation. Major emphasis was laid on enlisting community participation in one of these villages, Pudukuppam, where the malaria incidence was the highest. The results of the study are presented here.

Study area: Pondicherry (11°-56°N and 79°-53°E) situated on the Coromandel coast of penisular India, is a former French colony with many villages distributed along 23 km of sandy coastal belt. Four coastal villages *viz.*, Nallavadu, Naram-bai, Pannithittu and Pudukuppam with population of 1400, 750, 1275 and 800 respectively, were selected. Situated well within the tropics these villages experience a hot and maritime type of climate characterised by small daily range of temperatures, humid weather and moderate rainfall. The advancing southwest monsoon (June-Sept.) brings about 362 mm rainfall (average for two decades) spread over 21 days. The retreating north-east monsoon (Oct.-Jan.) is the principal rainy season which brings about 713 mm (average for two decades) distributed over 28 rainy days. There are however, yearly fluctuations in the pattern of rainfall and the area, generally, is characterized by either drought conditions or very heavy rainfall in recent years. The inhabitants of these villages are mostly fishermen. Cattle are generally not domesticated. *Casuarina equisetifolia*, an important cash crop is

cultivated extensively all along the sandy coastal terrrain of Pondicherry. This is one of the easily grown cash crop which is extensively used as firewood by the people of this area. The area under cultivation is being extended every year, as planting casuarina crop serves not only as wind breakers but in preventing erosion of land by the sea. The plants need watering only during the initial stages until their tap roots reach the high subsoil water level in coastal areas. This necessitates digging of innumerable pits every year in the plantation. These pits are shallow (2-3m deep) and form a perennial mosquito breeding source. There were 329 pits enumerated within 1 km operational area, at the commencement of the study, and this number gradually increased to 502 within three years. In addition to such pits, three of the four villages have vast expanses of backwaters which support prolific breeding of many mosquito species. The mosquito density always remained high and indigenous transmission of malaria was an annual feature.

Malaria situation in relation to socio-economic background :

(*i*) Occupational reasons—It was observed that the migratory pattern of the fishermen community has a direct bearing on the malariogenic conditions in these villages. The fishermen visit many endemic areas such as Rameswaram, Vizagapattinam, Nagapattinam, Thondiarpet, *etc.*, during the course of their fishing activities, invariably contract malaria and return sick to their native villages where they form the foci for further local transmission. Locating positive cases at a given time is also a formidable task as the menfolk go to the sea at all odd hours.

(*ii*) Improper and inadequate treatment—Due to the easy availability and indiscriminate use of chloroquine many cases escape detection and radical treatment. Even when positive cases are detected, the delay in the commencement of radical treatment enhances the chances of local transmission. In many cases it was difficult to persuade the patients to take full course of treatment, as the fever subsides after the initial dose. Side-effects of the antimalarial drugs such as giddiness, nausea, *etc.* also prevent the rural people from taking the complete course of treatment. Primaquine and chloroquine wrapped in paper which were handed over to a relation or neighbour by the malaria worker could be seen kept safe in the roofs of their dwellings for months together.

The socio-economic background of the study villages : The coastal fishermen villages in this area generally lack basic amenities like drinking water, proper housing, communication system *etc.* Due to illiteracy, poverty, deep-rooted superstitions and unhygienic living conditions, they also suffer from a variety of other illness/conditions like malnutrition, gastero-enteritis, tuberculosis, *etc.* The pattern of life in villages is guided by the locally elected '*Panchayat*' which is controlled by the affluent few. The village *panchayat* enjoys vast powers including the power of extradition of individuals who question their authority (as it happened in one case). All disputes among the villagers are settled by the *panchayat.* Governmental aid is invariably routed through this body and no governmental agency approaches an individual directly. Similarly, no villager could approach a governmental department directly without getting the approval of the *panchayat.* Even

murders and suicides committed within the village can be probed, only if the *panchayats* co-operate with the investigating agencies.

Under such conditions, it is a formidable task to penetrate into the system and win the confidence of the villagers and to make them participate in vector control programme. The villagers have their own priorities. They are indifferent to plans which are usually made without consulting them and ignoring their views on how best they want to be helped. Often these plans are made without any knowledge of the village needs. Many times such plans are forced upon the villagers and the latter acquiesce passively to their implementation, without participating in them. Their acquiescence is often mistaken for cooperation/participation. The impact of the welfare measures is not really felt by the majority of the villagers, as a result. Visits by Government officials often accompanied by their minions in the department, attract curious onlookers. Being traditionally hospitable, the visitors are entertained. Many promises are made with regard to the numerous grievances of the villagers, which are rarely fulfilled. The whole governmental programme thus gets a bad name. As far as the villagers are concerned, the VCRC exercise was in no way different from any other governmental visits and therefore, initially, there was considerable indifference and resistance too. It is well known that no programme will succeed unless the community co-operates and participates in its execution[3]. Further, "No Government can change the habits of people without their hearty co-operation and if the latter is forthcoming government will have to do little in the matter[4]". In the absence of a clearcut method to enlist community participation in any programme, the VCRC proceeded on a trial and error basis to achieve community participation in vector control programmes. It took nearly two years for the VCRC to gain the confidence of the villagers and to make them accept the programme. A community can not be easily motivated to willingly participate in a programme of vector control from which the gains are indirect. They co-operate only with such agencies which brought direct and perceptible benefits to them like agriculture, fisheries, animal husbandry departments and money-lending agencies such as banks.

Since the village community consists of different castes, religions and political groups, it is not an easy task to identify a proper leader who is a representative and is acceptable to the community as a whole. Hence the western model of selecting opinion leader or group leader does not work in such situations. Group rivalries are so serious in some villages that, any welfare measure in which one group is involved is bitterly opposed by the other. Even though in coastal villages the inhabitants are mainly fishermen, the group rivalries are based on economic status and political shades. During the initial stage of the VCRC operations, youth clubs and *Madhar Sangams* (women's organization) were formed in some villages and the response was very encouraging in the begining. In order to maintain their enthusiasm many incentives such as sports articles, newspapers, magazines, musical instruments *etc.* were provided through the Block Development Office. In Pudukuppam, the 'leaders' were afraid that the youth, mainly from the

lower income strata, may rebel against them once they are organised and allowed to function independently from their control. The 'leaders' rebelled and ultimately the youth clubs had to be disbanded. In the process, the whole village turned hostile to VCRC as an "organization out to disrupt the social fabric" of the village. Hurdles were created, abuses were hurled and an atmosphere was created in which the very functioning of the VCRC in the village appeared to be difficult. All these happened because the VCRC directly approached the poorer sections of the community without analysing the background and functioning of the village system as a whole. From then on, the village *panchayat* leaders were coaxed and cajoled and the VCRC persisted in its activities in this village and started helping the *panchayat* in carrying out many welfare measures such as laying an approach road with the help of the local government administration, rectification of electric lines *etc.* without talking about vector control. Slowly and gradually, the villagers were convinced that the VCRC was genuinely interested in helping them. Once they were convinced, things changed for the better and the village community, in spite of internal group rivalries, whole-heartedly rallied and co-operated with the VCRC. Living as one among them and sharing their problem was found to be the ideal way to penetrate into the village life. In all village functions, whether that of an individual, family or of the community, the VCRC was invited by the villagers and this was reciprocated by the VCRC, which earned the goodwill of the villagers.

The VCRC generally acted as their liaison agent with other governmental agencies for the redressal of their grievances; problems with drinking water were solved on a priority basis and the VCRC helped to get a borewell and pumping station installed by the local administration for Pudukuppam village which was the worst affected. Other needs like provision of approach roads, electricity, cremation ground *etc.* were also attended to by the VCRC. Many a time, the services of VCRC was requested for rectification of leaking/clogged pipelines, cleaning of overhead tanks and wells *etc.* which were also attended to by the VCRC in all study villages. Weekly visits of doctors from the Primary Health Centre to cater to medical needs were also arranged. All minor ailments were attended to daily by the VCRC team at their doorstep. By and by, discussions were held on health needs with the help of educational films. In some villages, garbage accumulated over many years, which supported prolific breeding of flies were removed and buried by the villagers. However, it was realized that to ensure willing community participation in vector control programme it is not a one time affair by the villagers donating free labour and that such enthusiasm will not last long. Hence a massive door-to-door health education campaign was carried out to create an awareness regarding vectors and vectorborne diseases and the importance of the involvement of the people in the control operations. The health education activities were primarily directed to foster activities that encourage people *to want* to be healthy, *to know* how to maintain health, *to do* what they can individually and collectively and *to seek* help when needed[5].

When vector control strategies are designed for rural areas, the criteria

suggested (C.P. Pant, personal communication) are that the technology works, it fits into the hands and minds of the people, the community can afford it within the resources available, it can be extended and built upon, and it should be environmentally harmless.

The villagers view the different aspects of their daily life as a whole and do not view health care in isolation. Only if and when all other needs are satisfied, they will direct their attention to health care and that too only when the health problems are serious. In spite of catering to their needs in all possible ways the active participation of the village community in vector control measures such as collection and introduction of larvivorous fishes, emptying containers breeding mosquitoes once a week, *etc.* could not be achieved on a long term basis. It should also be realised that as a vast majority of rural population live below the poverty line, their main concern is to find ways and means for their livelihood and in this process, they may not have time for vector control from which there is no direct monetary gains. However, no vector control programme can be sustained for long in a village community by Governmental efforts alone. It was also apparent that the village community would not continue the simple environmental method of vector control, once VCRC left the scene. It was at this stage the strategy was modified to link the vector control operations with developmental activities in which the villagers derive direct financial benefits, so that they will continue to carry out the programme on a permanent basis. It was by sheer accident rather than due to any deliberate planning that major steps for commercial exploitation of algae and prawn culture were undertaken to directly involve the community in the programme.

Economic exploitation of algae which support mosquito breeding in backwaters : A large area of backwaters in the three study villages continued to be a perennial source for mosquito breeding except for a very short spell during monsoon when the larvae get flushed out. Eventhough many species of predatory fishes are present in backwaters, effective predation is not possible, due to extensive growth of aquatic algae, *Enteromorpha compressa* var. *pinqulata* and *Chaetomorpha littorea* (Family : Chlorophyceae) which not only shielded the mosquito larvae from predators but also formed oviposition sites. Since larvivorous fishes are not found effective in controlling mosquito breeding in the presence of algae and other alternate methods such as application of chemical insecticides is not practical because of the vastness of the area, manual removal of algae was initiated from July 1981 onwards. During the course of four months from July to November about 130 tonnes of algae, covering approximately 34,075 square metres and involving 1974 man hours, were removed from back waters of Pudukuppam alone. With the onset of monsoon the algae were submerged due to rise in water level and high wave action also prevented mosquito breeding for a short period. After heavy rains some portion of the backwater became contiguous with the sea. However, during the post-monsoon season when the water level receded, the luxuriant growth of algae made this habitat again highly mosquitogenic.

In order to find a permanent solution for the removal of algae, the possibility of economic utilization of the material

was explored in the VCRC laboratory. It was found that *E. compressa* could be used in the preparation of card boards. Various entrepreneurs engaged in paper manufacture were approached to utilise this algae as a raw material in their products. A mottled art paper was prepared using this algae by the handmade paper unit of Aurobindo Ashram, Pondicherry, which found a ready export market. It was also found that using algae as a raw material was very economical. Some other industries also carried out trials and found that this algae could be used in making file covers, card boards *etc.* A society has already been formed by the local villagers for the collection and marketing of this algae. The economic exploitation of algae has resulted not only in controlling mosquito breeding in habitats infested with the algae but also provided employment opportunities for the village youth. The society made about Rs. 5,000 ($ 500) during the pre-monsoon period each year. The local Government have also shown some interest in setting up of a cottage industry in the vicinity of these villages for the manufacture of papers, card boards, *etc.* The development of this technology has paved the way for vector control operations by the villagers themselves on a permanent basis, coupled with employment opportunities for them.

Conversion of backwater area into a prawn culture pond : It was found in certain backwater regions that when water level recedes into small pools, mosquitoes breed prolifically in them. It was observed that prawns could breed and survive in such habitats and the villagers could earn a considerable money, as prawns are expensive. After discussion with the villagers, a part of this area was deepened into a prawn culture pond and many small pools which were highly mosquitogenic were eliminated. The villagers, realizing the beneficial aspects of this project, not only contributed labour by sending one man from each family, but the *panchayat* also spent more than Rs. 3,000/- for hiring additional labourers. The VCRC provided only the leadership and encouragement. The whole operation took about 20 days. Approximately 28,000 cubic metres of mud was removed from the demarcated one hectare area and a channel 150 m long 7 m wide and one meter deep was also provided to drain out the water from the adjoining areas into the prawn culture pond. This project eliminated a large number of small pools breeding mosquitoes and helped in filling of adjoining low lying areas. The yield from the sale of prawns was decided to be shared by the village panchayat, and they have also agreed to initiate many more schemes from the profit derived. Conversion of such a mosquitogenic area into a prawn culture pond provided annual financial returns of about Rs. 10,000 to 20,000 depending on the yield.

Vector control operations in other habitats : In approaching the problem of malaria vector control, major emphasis was laid by the VCRC on the environmental modification aimed at preventing/eliminating or reducing the larval habitats. The mosquitogenic habitats in the villages under study were casuarina pits, backwaters, coirpits, containers and earthworm pits. *Anopheles subpictus*, the coastal form of which has been incriminated as the vector of malaria in these villages[6], was the most predominant species breeding in these habitats. Since the casuarina pits cannot

be closed down without affecting the economy of the villagers, indigenous larvivorous fishes were introduced in these pits. These habitats were checked every week and wherever fishes were absent they were re-introduced. Mosquito control was achieved in 96 per cent of such pits using *Gambusia affinis*[7]. Though the fish is present in large quantities in nearby ponds, the villagers could not be persuaded to seed the casuarina pits with fishes even though the efficacy of the fish in controlling breeding was clearly demonstrated to them. Being relatively shallow habitats, no edible fishes could be grown in this habitat.

Coir making is a cottage industry in coastal villages as the fishermen need coir ropes for fishing. They ret coconut husk in many earthern pots in each house, which breed mosquitoes. Emptying of these pots was attempted and discontinued because the villagers have to travel a long distance to fetch water to refill the containers. Covering the pots with nylon netting was tried and was found effective. However, the villagers did not always keep the pots covered, once they were emptied. Release of *Toxorhynchites splendens* larvae in these containers were made. Drastic reduction could be achieved in the container breeding mosquitoes as a result[8]. Many pits dug by the villagers for collecting earthworm for use as fish bait were closed by the people. Many villagers, especially children, learnt to recognize mosquito larvae and they were taught to eliminate small breeding places by filling. Over 400 such pits were closed by the villagers. They were also taught to dig large pits for retting coconut husks and close them with coconut and/or palm leaves on the top and cover them with sand so that these pits are not used for egg laying by mosquitoes.

Conclusion

While many simple methods are available for vector control in different habitats, it is an uphill task to persuade the villagers to do this work on a continuing basis. The methods suggested for control should be easy to implement and within the means and capacity of the villagers. Except during an epidemic when the majority of the villagers suffer, they cannot be expected to devote their time at the cost of their other daily chores. The exploitation of algae and culture of prawns in some areas were profitable for the villagers and vector control was only a by-product of this activity. Such situations may not exist in many villages. Therefore, the examples given here are not replicable. What is important is the approach tried *i.e.* the attempt made to integrate vector control with the overall socio-economic development. Health care delivery *per se* is not perceptible, unlike the construction of a bridge, roads, giving milch cows, loans to buy fishing nets *etc.* But if the activities of the different agencies of the health care delivery can be coordinated and if vector control and environmental sanitation can be integrated with this, it will make a much better impact on the villages. The latter must become conditioned to live a disease-free life at least for some years so as to make them realize the importance of good and comprehensive health care delivery. This is what is now being attempted by the VCRC in a scheme covering all the 21 villages under a Primary Health Centre.

Acknowledgment

The hard work and dedication of all the staff engaged in the project is gratefully acknowledged.

The co-operation rendered by the Health and Family Welfare Services, Government of Pondicherry, is also duly acknowledged.

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Indian J Med Res 80, July 1984, pp 125-126

Book Review

Treatment of infective endocarditis, Alan L. Bisno, Ed. (Grune and Stratton, New York) 1981. 340 pages. Price : $ 38/-.

This book offers the proceedings of a conference on 'Treatment of infective endocarditis' held by the Committee of Rhumatic Fever and Infective Endocarditis of the American Heart Association in April, 1980. The aim of the Conference was to survey all aspects of the treatment of infective endocarditis and provide an assessment of the present state of the art. The Editor and other contributors have largely achieved this aim, since the book serves as a comprehensive treatise on current knowledge not only of the medical treatment of infective endocarditis but also various aspects of its diagnosis.

While the first chapter gives a short historical review, the next three chapters present details of the management of *Streptococcus virdans* endocarditis and the results expected with optimal therapy. The advantages of combined therapy, with penicillin and streptomycin are highlighted although the optimal duration of therapy appears to be still controversial. The succeeding chapters highlight the microbiological and therapeutic aspects of enterococcal endocarditis, a less common but more problematical form of the disease. The importance of combination therapy with a two antibiotic regimen is obvious and the results quite satisfactory. The chapter on prosthetic valve endocarditis is of great relevance to the current clinical scene. The importance of early diagnosis, inherent difficulties in achieving this, the role of echocardiography as a diagnostic procedure and the importance of aggressive antibiotic therapy with surgical replacement of the prosthetic valve at an early stage of disease, if required, are well brought out. Of equal interest are other facets of the surgical treatment of infective endocarditis ably described by Dr Bruce Rentz and his colleagues focussing on the changing view points regarding treatment of infective endocarditis in the present day setting, emphasizing the important role being played by surgery in prevention as well as treatment of serious and potentially fatal complications like embolisation, progressive congestive heart failure due to valve dysfunction, aneurysmal rupture *etc*. A special discussion on the treatment and prevention of infective endocarditis in infants and children highlights the problems encountered and their management in this vulnerable group. The last chapter updates our knowledge regarding indications and recommendations for prophylaxis against infective endocarditis.

While, in general, the quality of presentation and discussion are excellent, in common with other multiauthored books, this also suffers from inconsistency and lack of uniformity in style, and from considerable degree of repetition. Discussion of the special problems of infective endocarditis in the elderly is conspicuous by

its omission. Diagnosis and management of culture negative infective endocarditis have also been handled only perfunctorily.

In spite of the shortcomings listed, however, this publication is recommended as a useful reference book for all those interested and involved in managing patients with infective endocarditis.

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ANNOUNCEMENTS

The Anophelines of India

by

Dr T. Ramachandra Rao

This treatise authored by one of the foremost malariologists of India is intended to be a source book of comprehensive and up to date information on the biology of anopheline mosquitoes in India and neighbouring countries. Aimed at strengthening the basic research on malaria and its vectors, this book offers detailed information on the Systematics, Distribution, Genetics, Insecticide resistance and all related aspects of biology of 51 anopheline species prevalent in the Indian subcontinent. Besides information on taxonomy and distinguishing characters of each species, this treatise gives a comprehensive appendix providing general keys for the identification of the adults, larvae and eggs of the anophelines. A very useful book for all entomologists and malaria researchers.

Price Rs. 150·00; $ 45·00

For obtaining copies, draft/cheque drawn in favour of the Director-General, Indian Council of Medical Research, may be sent to the Chief, Division of Publication and Information, Indian Council of Medical Research, Ansari Nagar, New Delhi 110029 or Director, Malaria Research Centre (ICMR), 22 Shamnath Marg, Delhi 110054.

* * *

The Vector Control Research Centre, Pondicherry, will undertake to identify blood meal source of mosquitoes and other haematophagous arthropods free of charge. Blood meals will be tested against 13 mammals including man and nine bird species. More species are being added to the antisera bank. Research workers may avail this offer by sending filter paper strips of blood meal or fed arthropods in gelatin capsules to the Director, Vector Control Research Centre, Pondicherry 605006.

NOTES & NEWS

Community participation and vector control

That it is possible to achieve success in vector control of malaria by active community participation in a rural area has been effectively demonstrated by a pilot study carried out by the Vector Control Research Centre (ICMR), Pondicherry in 4 coastal villages of Pondicherry. The major findings of this project which are presented in this issue of the Journal [*Indian J Med Res* **80** (1984) 117] highlight the classic scene as existing in a coastal tropical village growing of Casuarina trees and the resultant multiplication in the water pits, the perennial mosquito breeding sources. With persistent efforts and health education, community participation was achieved through which the water logged areas containing algae could be commercially exploited. The algae was used for cardboard making and in addition to this, the villagers were helped to culture prawns that breed and survive in the villages. The project thus helped not only containing malaria but encouraging the villagers to involve themselves in financially beneficial rural industries in a small scale with practically no investment. The VCRC now proposes to extend this successful scheme to other villages in the area.

* * *

Infantile bloody diarrhoea and cow's milk

Many paediatricians have thought the bloody diarrhoea associated with cow's milk in infants to be a mere allergy and would go away if ignored studiously. However recent evidence [Jenkins *et al.*, *Arch Dis Child* **59** (1984) 326] has provided a new insight by labelling the condition colitis rather than gastro-intestinal allergy. The major difference with the ulcerative colitis of adults is that in the adult form there is little evidence that it responds to dietary measures in sharp contrast to the infant colitis.

* * *

Dust allergy and birth dates

An interesting coincidence has been noticed between dust mites and birth dates of asthmatic patients. The report [*Clin Allergy* **13** (1983) 529] claims that patients with asthma and positive skin tests to house dust mites are more often born in summer and autumn months. Since dust mites are more abundant between May and September these data provide additional support to the clinical importance of exposure to the allergin in the first six months of life to dust mite allergy. Allergin specific IgE antibodies are most readily induced in the first few months of birth.

* * *

Coronary heart disease

Coronary heart disease has been less fatal in the United States in recent years [*Am Heart J* **106** (1983) 783]. The report says that death rates are falling though with a pronounced regional variations. Women and blacks particularly have less mortality rates as also the nearly one half decrease in the sudden death rates. Perhaps, the authors feel, this can be correlated with an overall decline in consumption of cigarettes, lowered blood pressure and lowered cholesterol levels.

Workshop on Biomedical Information Science

A Workshop on Biomedical Information Science was organized by the Indian Council of Medical Research, New Delhi, for the Council's librarians during April 17-19, 1984. Apart from five Indian Information Scientists, two Information Specialists from Karolinska Institute, Stockholm constituted the faculty for the Workshop.

A number of Senior Librarians and Documentation Officers, incharge of the Council's various Institutes participated in the Workshop. The participants were exposed to an intensive course on biomedical information collection and retrieval with particular reference to the MEDLARS data base of the National Library of Medicine, Bethesda, USA, and the Karolinska Institute, Stockholm.

* * *

Director-General, ICMR honoured

Prof. V. Ramalingaswami, Director-General of the Indian Council of Medical Research, New Delhi, has been formally admitted as a member of the USSR Academy of Medical Sciences. He is the first Indian Medical scientist to be elected to the prestigious Soviet Academy.

Next issue

Tuberculosis prevalence survey in Kashmir Valley
S. Mayurnath, D.S. Anantharaman, G.V.J. Baily, M.P. Radhamani R.S. Vallishayee, P. Venkataraman & S.P. Tripathy

Typing of herpes simplex virus isolates by different serologic & biologic methods
Gita Sitapathy, Pradeep Seth & S. Balaya

Spontaneous loss of drug resistance under laboratory conditions in clinical isolates of *Escherichia coli*
J.N.S. Yadava, M.Q. Ansari, Reeta Goel & H.B. Rajani

Proteus infections with references to proteocine typing
Savitri Sharma, R. Sambasiva Rao & V. Ravi

Lymphocyte proliferation & intestinal absorptive functions in mice infected with *Giardia lamblia*
V. Radhakrishna, N.K. Ganguly, A. Mahmood & R.C. Mahajan

An effective intervention to reduce the prevalence of anaemia in children
Subadra Seshadri, Kalpana Hirode, Pallavi Naik, Alka Shah & Neeta Gupta

Anabolic steroids in aplastic anaemia
Vinod Kochupillai, Subhadra Sharma & K.R. Sundaram

Nutritional support as an adjunct therapy of advanced cancer patients
S.P. Tandon, S.C. Gupta, S.N. Sinha & Y.P. Naithani

Ectopic secretion of human chorionic gonadotropin by gynaecological tumours
S.K. Gupta, K. Buckshee & G.P. Talwar

Use of monoclonal antibodies to define peripheral blood lymphocyte subsets in systemic connective tissue diseases
A.N. Malaviya, Premavathy Rajagopalan & R. Venkitaraman

Dignostic efficacy of haemoglobin A_2 in heterozygous β-thalassaemia
A.K. Saraya, Rajive Kumar, V.P. Choudhry, R.S. Tyagi & A.K. Sehgal

Foetal loss & family size in neonatal jaundice due to ABO-HDN G6PD deficiency & low birth weight
Snehalata C. Gupta & H.M. Bhatia

Effect of mustard & peanut oil on the regression of experimentally induced aortic & coronary atherosclerosis of monkeys
R.S. Kukreja, B.N. Dutta, A.K. Sarkar & R.N. Chakravarti

Efficacy of a commercial rat feed & three other constituted diets in sustaining the body growth of rats
Carol Mascarenhas, B. Rajanna, K. Muniyappa & T. Desiraju

Next issue

Profile of non-organ specific autoantibodies in thromboangitis obliterans
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Ambika Nanu

Absorption of acetylcholine in the intestine & the eye
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T. Mohan Kumar, Manuel Gimenez & Paul Sadoul

Immunoglobulins in gastric aspirate before & after oral cimetidine in certain gastric lesions of acid secretors
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